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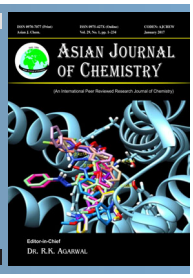

Recent Advances and Applications of Turbulent Flow Chromatography

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Turbulent flow chromatography is a separation technique combined with online extraction of macromolecules in biological samples. Turbulent flow chromatography was basically developed to separate macro molecules from small molecules and analytes without any pretreatment prior to injecting the sample onto the chromatographic system, hence it reduces the time of analysis and also bypasses the manual extraction step of macro molecules in the biological samples. It finds numerous applications as an on-line sample cleanup of biological matrices in preparation of sample and extraction of analytes from biological matrices during estimation of analytes in biological samples and bio-analytical method development. The present work describes the advancements, its wide range of applications as well as advantages and disadvantages of the turbulent flow chromatography over the existing methods.

Keywords: Turbulent flow chromatography, On-line extraction, Bio-analytical.

INTRODUCTION

Turbulent flow chromatography (TFC) was introduced in the late 1990s as a technique for direct injection of biological fluids on to a column packed with 50 μm spherical porous particles [1]. Turbulent flow chromatography is generally associated with liquid chromatography tandem mass spectrometry (LC-MS/MS), which is well established in the pharmaceutical industry as the primary analytical technique to determine drug concentrations in biological matrices [2].

While developing the analytical methods the preparation of samples for determination of analytes in biological samples or samples containing macromolecules is the critical step and it also needs long analytical runs. The recent implementation of automated (on-line extraction) turbulent flow chromatography (TFC) has allowed fast sample clean-up and partly removed the bottleneck associated with sample preparation. Turbulent flow was first defined over 100 years ago by British physicist Osborne Reynolds [3]. Reynolds [4] discovered that the flow of a fluid through a conduit becomes turbulent when the momentum of the fluid exceeds its resistance to flow by a factor of 2000-3000. The ratio of these opposing forces, known as the Reynolds number (Re), is expressed in eqn. 1:

$$Re = \frac{\text{Inertial forces}}{\text{Viscous forces}} = \frac{u\rho d_p}{\mu} \quad (1)$$

where u is the linear velocity of fluid, ρ is the density, d_p is the particle diameter and μ is the dynamic viscosity of mobile phase.

Thus, turbulent-flow systems take the advantage of large, porous particles (*e.g.*, 20-60 μm), packed into narrow bore columns (b1 mm i.d.), run at high flow-rates to achieve the minimal required value for the Reynolds numbers, where the flow changes from laminar to turbulent. Interestingly, it has been noted that several authors have claimed to operate in the region of turbulent flow, in view of the above mathematical distinction of turbulent flow, they were probably operating somewhere in the transition zone between purely laminar and a purely turbulent-flow [5,6].

Early attempts to decrease analysis time in chromatography have been blocked by the existence of an optimum mobile phase velocity and consequently of an apparently fixed analysis time for a given chromatographic system. It was, however, soon pointed out [7] that the analysis time could be shortened by increasing the mobile phase velocity and increasing the column length to compensate for the accompanying deterioration in the column efficiency.

The routine usage of hyphenated techniques for quantification has provided a highly selective means of monitoring the peak of interest without the need to develop a lengthy chromatographic method. With minimal sample preparation and short analytical run times (*e.g.* less than 2 min), the original complexity associated with biological samples have gradually been addressed.

The properties of fluid flow and mass transfer within it, is a vast type of study [8-12]. Reynolds number is one of the most important parameter used to characterize the flow of fluids [4]. The Renolds number is defined as the ratio of the inertial to viscous forces present in a fluid system which depends on the system being investigated. To characterize the flow of a fluid one must to identify the proper length scale of flow up to which it is characterized. Thus the Reynolds number definition is different in an open tube to that obtained for a fluid flowing in a packed bed.

For the laminar flow system it is comparatively easy to define an exact equation for the parabolic flow [13], but in a turbulent system it is more difficult to define an exact equation for same. The flow pattern depends upon flow rate. The flow profile describes variation in the dispersion coefficients of the analyte and the variation in velocity profile. The flow profile in turbulent flow system is much flatter than in laminar form, since the dispersion coefficients are different for both the flow patterns. This results in greater mass transfer within the mobile phase, which reduces band broadening [8].

Theory

In chromatographic separation one must know about the role of optimum flow rate to observe the band broadening processes. As early as 1956, van Deemter identified the three major contributing factors in band broadening in chromatography are Eddy's diffusion, longitudinal diffusion and linear velocity. The relation between these factors and band broadening is represented in van Deemter equation [14].

$$HETP = A + \frac{B}{v} + Cv \quad (1)$$

where, HETP = Height equivalent to theoretical plate; A = Eddy's diffusion; B = Longitudinal diffusion; C = Mass transfer coefficient; v = Linear velocity.

The ideal chromatographic system shows that the components present in separation mixture can form discrete narrow bands based upon their nature when they diffuse through the column. The column efficiency depends upon band narrowness which depends upon the time spent by the components in the column while the resolution is determined by the ability of column to separate individual components of the original sample. This value can be quantified by a parameter referred to as the plate number or number of theoretical plates, N. If the resulting peak is Gaussian in form, then the following eqn. 2 is a good approximation of the efficiency of the column.

$$N = 5.54\left(\frac{t_r}{W_{1/2}}\right)^2 \quad (2)$$

where, t_r - Retention time of compound; $W_{1/2}$ - Peak width measured at half the peak height; N- Number of theoretical plates.

A plot of the HETP *vs.* flow rate produces a parabola, which indicates that the best smallest reduced plate height or HETP can be achieved at optimum flow rate for most efficient separation (Fig. 1). A HETP of 2 is generally accepted as being very good, with values up to 10 being acceptable, for a standard HPLC column. Better efficiencies can be achieved using different chromatographic techniques but they differ in various methods of driving the mobile phase through the column.

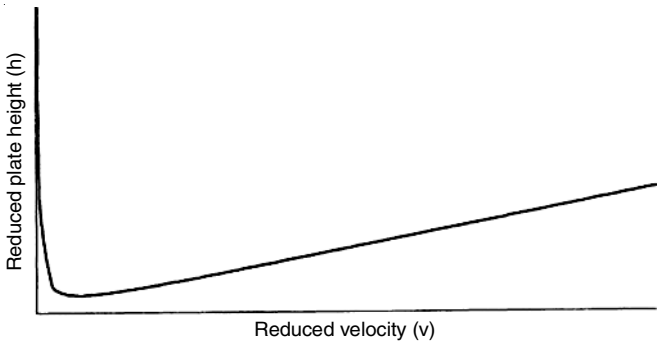

Fig. 1. van Deemter plot showing optimum reduced velocity, giving minimal band broadening which occurs at lower reduced velocities

The concepts of turbulent flow chromatography can be well understood by observing an open tubular system and then the concepts transferred to a packed bed system. Golay's equation [15] explains the phenomena involved in open tubular chromatographic system under laminar flow conditions. Under these conditions a parabolic radial velocity profile can be assumed which indicates diffusional processes dominate mass transfer within the mobile phase. However under turbulent conditions the radial velocity profile is no longer parabolic (Fig. 2) but becomes velocity dependent [13] and radial transfer is dependent on convection due to the spontaneous formation of eddies, although diffusional processes still do occur within this environment.

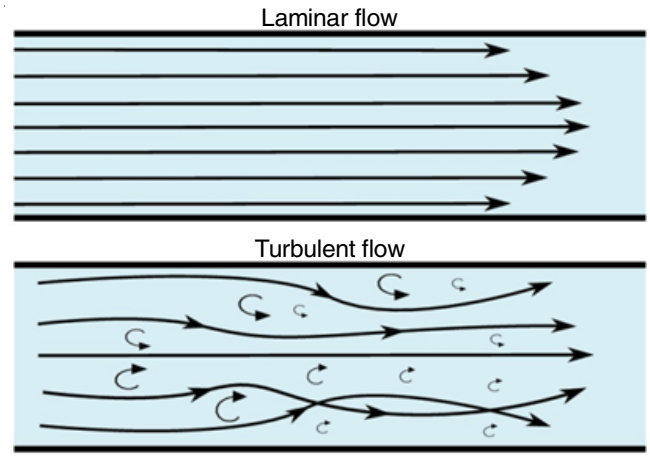

Fig. 2. Difference between laminar and turbulent flow

Turbulent flow occurs as a result of particles becoming overwhelmed by the stationary phase and is more common in column chromatography.

There have been several studies of band dispersion in turbulent flow [16-18]. Early work indicated how turbulence affected the mass transfer [7] and although computational

models have developed substantially since these early days, they are still applicable and give a good understanding of how mass transfer effects are substantially reduced by moving to a turbulent flow regime.

Principle

Turbulent flow chromatography (TFC) is a high-throughput sample preparation technique that uses high flow rates (4-6 mL) through column packed with sorbent particles with large pore sizes (30-60 µm) [19]. Moderate back-pressure is developed in the column due to large pore size, so the column can serves as both extraction and analytical column. By applying higher flow rate conditions, solvent doesn't exhibit laminar flow but exhibits turbulent flow. This leads to the formation of eddies (Fig. 3) which promote cross-channel mass transfer and diffusion of the analytes into the particle pores.

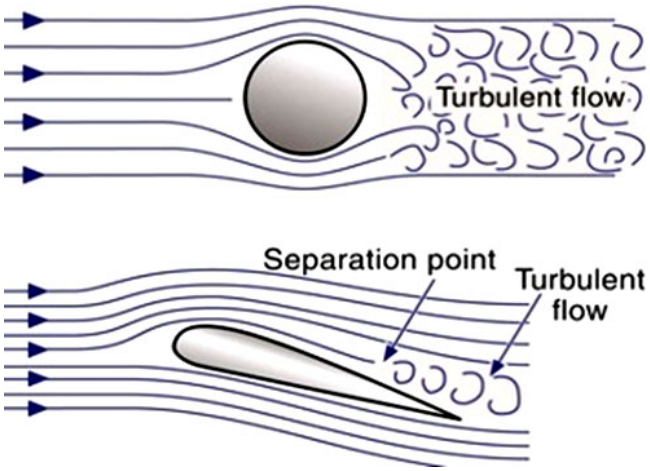

Fig. 3. Principle involved in turbulent flow chromatography

When the samples applied on to the column along with aqueous mobile phase (Fig. 4), the small molecules diffuse into the particle pores, more extensively than macromolecules (*e.g.* proteins, lipids, sugars). The macromolecules are drained to waste and they are unable to diffuse into the particle pores. Then the trapped analytes across the particle pores are desorbed from the turbulent flow chromatography column by back diffusing them with a polar organic solvent and the elute is passed through HPLC column (normal low flow rate) with a switching valve, for further separation and subsequent detection (usually by MS/MS). For every sample analysis turbulent flow chromatography column is reconditioned and primed.

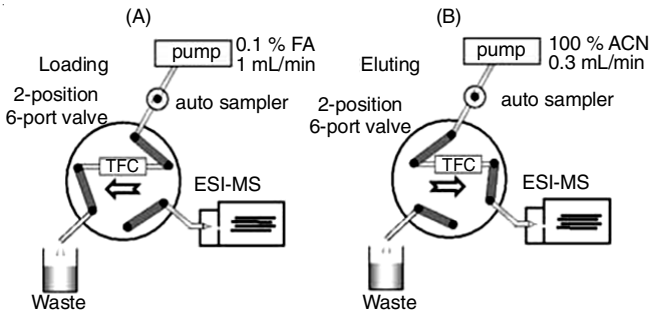

Fig. 4. Process involved in turbulent flow chromatography separation

Instrumentation: Simple HPLC pumps and switching valves can be used to carry out turbulent flow chromatography, although specialist equipment, termed high turbulent liquid chromatography (HTLC), is also available. The columns used for turbulent flow chromatography contain common HPLC sorbents but of larger particle sizes. The chromatographic efficiency of turbulent flow chromatography is similar to that of laminar flow but at much lower flow rates (Fig. 5). Turbulent flow chromatography is also effective at separating residues that are bound to sample proteins. The use of turbulent flow chromatography eliminates time-consuming sample clean-up in the laboratory and results in a much shorter analysis time, higher productivity and reduced solvent consumption without sacrificing sensitivity or reproducibility.

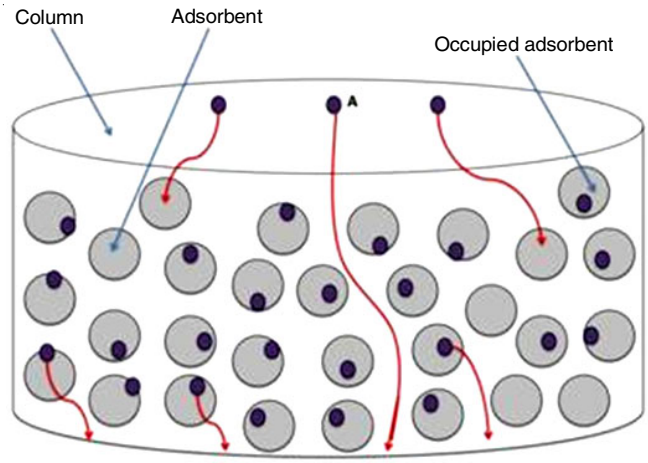

Fig. 5. Typical turbulent flow chromatography column

The turbulent flow chromatography system includes components as same conventional liquid chromatography system, additionally it is composed of turbulent flow column. Fig. 6 shows the block diagram of turbulent flow chromatography system.

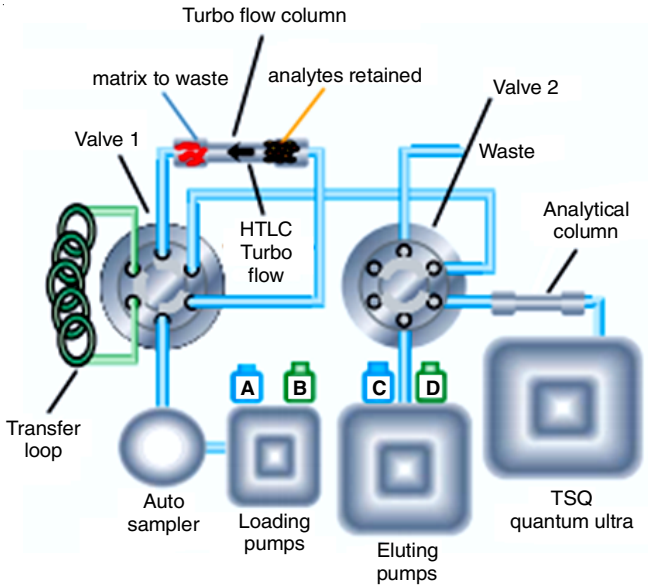

Fig. 6. Typical diagram of turbulent flow chromatography

Advantages: Turbulent-flow chromatography coupled with tandem mass spectrometry (TFC-LC-MS-MS) has

been described as a technique that eliminates time-consuming sample clean-up and increases productivity with good sensitivity [1]. Nowadays, turbulent flow chromatography can be used as a high-throughput sample preparation technique that makes use of high flow rates in 0.5 or 1.0 mm internal diameter columns packed with particles in the size range 30-60 µm. These on-line methods have the advantage of direct injection and elution of the analyte, which removes the time-consuming off-line steps of evaporation, reconstitution and preparation. In addition to the time savings achieved using automated sample optimization and multiplexing, further utilization of the small injection volume required on the 0.5 mm i.d. columns could be exploited.

Disadvantages: Turbulent flow chromatography is often used for on-line sample cleanup of biological matrices in liquid chromatography-mass spectrometry applications. However, the general mechanisms are not well represented in the literature and there is a lot of misunderstanding of turbulent flow chromatography's basic principles.

Applications

A number of investigators have reported the successful application of turbulent flow chromatography in different fields such as:

Pharmaceutical analysis

Estimation of enrofloxacin and its metabolite ciprofloxacin in edible tissues [20]: A mixture of acetonitrile, water and formic acid was used for extraction of tissue samples. The internal standard solution was added to an aliquot of the extract prior to injecting into the turbulent flow chromatography system. Polymer-based extraction column which is suited for pretreatment of samples at high flow rates can separate the matrix components contained in the injected sample from the retained analytes. The analytical column elutes and determines the analytes quantitatively using a tandem mass spectrometric detector.

Determination of antibiotics in environmental water sources [21]: Environmental and government laboratories had developed LC/MS methods to check the presence of antibiotics in water supplies. However, the pre-concentration and cleanup of samples was necessary to analyze the low-level concentration of antibiotics in environmental water sources. Bulk or large sample volume, is time consuming and reduces sample throughput. This turbulent flow chromatographic method that significantly decreases sample preparation time by applying on-line pre-concentration and extraction in conjunction with detection using the Thermo Scientific TSQ Quantum Ultra in highly selective reaction monitoring (H-SRM) mode for assaying antibiotics at low pg/mL concentrations.

Analysis of anti-infectives in wastewaters [22]: This work proves that the turbulent flow chromatography load columns significantly act as alternatives for on-line pre-concentration of waste water samples because they can be loaded at higher flow rates. The TCF prevents the samples from affect of fouling, thus decreasing analysis time and enhancing method robustness required for environmental polluents. Recoveries for the target analytes were between 86 and 141 %. Limits of quantification ranged from 45 to 122 ng L^{-1} and limits of confirmation from 37 to 142 ng L^{-1}.

Trace analysis of drug metabolites: Prediction of metabolic pattern is a crucial step in drug development new drug candidate in animals and humans. *in vitro* Metabolism studies include incubating the drug with sub cellular fractions, such as rat-liver microsomes (RLM). Metabolite produced by the microsomes should be precisely isolated, detected and identified using liquid chromatography with mass spectrometry. It was difficult to characterize trace amounts of metabolites without concentrating the large volume to small volume samples which is time consuming process (4 to 5 h). Turbulent flow chromatography (TurboFlow®) was successfully used to automate trace enrichment and separation of loxapine metabolites from an rat-liver microsomes preparation, while reducing the total analysis time down to 50 min.

Quantification of tricyclic antidepressants in serum [23]: First, human serum and an internal standard were injected directly onto a Cyclone-P online solid-phase extraction (SPE) column (0.5 × 50 mm). Following removal of serum proteins and other components of the analytes were passed through a Hypersil Gold C-18 (50 × 3 mm) turbulent flow analytical column. Elution occurred with a gradient of water and acetonitrile each with 0.1 % formic acid. Analytes were ionized and detected over a 3.5 min analysis time by electrospray-ionization mass spectrometry with selected reaction monitoring. Matrix effects were well-characterized and carry over, precision, linearity, recovery and limits of detection and quantitation were evaluated.

Determination of immunosuppressant's in transplant patients [24]: Total 1483 EDTA-blood pre-dosage samples from 147 kidney, 67 liver, 15 kidney/pancreas and 48 bone marrow recipients were collected. Turbulent flow chromatography was used for fast and efficient on-line matrix elimination after hemolysis and protein precipitation of 50 µL blood. Tandem mass spectrometric detection and quantification was performed using multiple reaction monitoring.

Analysis of chlorophenols in urine [25]: A Turboflow C18-PSPE column is used to online pre-concentrated to elute the analytes in back-flush mode and then separated on an AcclaimPA2 analytical column. Major parameters such as SPE column type, sample loading flow rate and elution time were optimized in detail.

Analysis of the mushroom poisons α and β-amanitin in human urine [26]: Poisonings with Amanita phalloides toxins need to fast diagnosis to avoid expensive and unnecessary therapies. After simple dilution and centrifugation of the urine sample, a fast on-line extraction using a Transcend TLX-II system based on turbulent flow chromatography (Turbo Flow) was established. A new Turbo Flow mode was introduced, the pseudo quick elute mode (PQEM), which had more options for method optimization than the generic quick elute mode (QEM). It allowed running several modes in one valve arrangement. The PQEM showed better practicability in routine and emergency analysis than the previously used methods. After extraction, the fast 15 min LC-high resolution (HR)-MS/MS analysis allowed reliable identification of α and β-amanitin based on fragments identified.

Estimation of citalopram, sertraline, bupropion and hydroxyl bupropion in serum [27]: Selected antidepressants

spike to serum, along with their corresponding isotopically labeled internal standards and they were subjected to protein precipitation. Samples were injected onto a turbulent flow chromatography column for on-line solid phase extraction and a Hypersil Gold C18 column for chromatographic separation. Detection was achieved using a TSQ Vantage mass spectrometer. Assay validation followed FDA bio-analytical guidelines.

Estimation of methotrexate and its metabolites 7-hydroxy methotrexate and DAMPA in serum [28]: Turbulent flow liquid chromatography (TFC-LC) combining positive heated electro spray ionization (HESI) is used for the determination of methotrexate (MTX), 7-hydroxy methotrexate (7-OH MTX) and 4-amino-4-deoxy-N10-methylpteroic acid (DAMPA) in serum. Methotrexate was isolated from serum samples (100 L) after protein precipitation with methanol containing formic acid and internal standard (MTX-D3) followed by centrifugation. The supernatant was injected into the turbulent flow liquid chromatography which is followed by electro spray positive ionization tandem mass spectrometry (TFC-LC-MS/MS) and quantified using a six point calibration curve.

Determination of verticine, verticinone and isoverticine in rat plasma [29]: Method uses an on-line extraction column (Waters Oasis HLB) and a fast HPLC column with sub-2m particle size (Agilent Zorbax StableBond-C18, 4.6 mm × 50 mm, 1.8 m) in a column-switching set-up were utilized. HLB is a reversed-phase extraction column with hydrophilic-lipophilic balanced copolymer (2.1 mm × 20 mm, 25 m particle size), which exhibits some turbulent-flow properties at a high-flow rate. The method combines the speed, robustness of turbulent-flow extraction, the sensitivity and separation efficiency of fast HPLC-MS to analyze multiple and trace constituents of selected compounds in plasma matrix. This method was successfully applied for pharmacokinetic study of verticine, verticinone and isoverticine, the chemical markers of *Fritillaria thunbergii*, after oral administration of total steroidal alkaloids extract of *F. thunbergii* to rats. Each plasma sample was analyzed within 7 min.

Determination of telcagepant in human plasma [30]: The diastereomer interference on telcagepant (MK-0974) determination during clinical study support can be eliminated by using on-line high turbulent-flow liquid chromatography (HTLC) methods. The on-line HTLC assays were achieved through direct injection of plasma samples, extraction of analyte with a Cohesive C18 column (50 mm × 0.5 mm, 50 m), followed by HPLC separation on a FluoPhase RP column (100 mm × 2.1 mm, 5 m) and MS/MS detection. The off-line SPE assay used Waters Oasis®HLB Elution plate to extract the analytes from plasma matrix before injecting on a FluoPhase RP column (150 mm × 2.1 mm, 5 m) for LC-MS/MS analysis. Under both on-line and off-line assay conditions, the diastereomer was chromatographically separated from MK 0974.

Analysis of perfluoroalkyl substances in cord blood [31]: On-line turbulent flow chromatography (TFC) in combination with tandem mass spectrometry used for the analysis of eighteen perfluoroalkyl substances (PFASs), in cord blood. A simple and rapid sample pre-treatment was optimized consisting on protein precipitation of 100 µL of sample with acetonitrile (1:1) followed by centrifugation during 10 min. The method was adapted to be sensitive enough and robust with minimum sample injection volume requirements (20 µL). The good applicability of this new approach was proved by the analysis of 60 cord blood samples from two different Mediterranean cities, Barcelona (Spain) and Heraklion (Greece). Ions perfluorohexane sulfonate (PFHxS) and perfluorooctane sulfonate (PFOS) were found at highest concentration.

Analysis of perfluoroalkyl substances in hair and urine samples [32]: The method was based on sample pre-treatment followed by online turbulent flow liquid chromatography and tandem mass spectrometry (TFC-LC-MS-MS) for analysis of 21 PFCs. The method was validated for both matrices. Percentage recovery was between 60 and 105 for most compounds in both matrices. Limits of quantification ranged from 0.1 to 9 ng mL^{-1} in urine and from 0.04 to 13.4 in hair. The good performance of the method was proved by investigating the presence of selected PFCs in 24 hair and 30 urine samples from different donors living in Barcelona (NE Spain). The results were indicative of bioaccumulation of these compounds in both types of sample. PFOS and PFOA were most frequently detected in hair and PFBA in urine.

Toxicological LC-MS screening for plasma sample analysis [33]: The sample extraction was carried by two Turbo Flow columns (Cyclone, C18XL) were connected in series. Aliquots of plasma samples were diluted with 10 µL of acetonitrile. Then centrifuge the mixture and 5 µL of supernatant was injected into the system. The Hypersil GOLD PFP column was used for LC separation.

Food analysis

Analysis of quinolones in honey [34]: Sample preparation involved simple dilution with H_2O followed by filtration and transfer of an aliquot into a vial. Sample extraction time was 4.5 min, while the overall analysis took 18.5 min. Recovery of the method ranged from 85 to 127 %, while the LOD of the method was 5 µg kg^{-1}.

Determination of antibiotics in milk [35]: Seven different classes of antibiotics (amino glycosides, sulfonamides, macrolides, quinolones, tetracyclines, lincosamides and trimethoprim) in milk were determined using turbulent flow chromatography (Transcend TLX system), directly coupled to a mass spectrometer (MS/MS). Samples were extracted/protein precipitated using acetonitrile, followed by centrifugation and filtration. Then extract was injected into the TLX-ESI-MS/MS.

Determination antibiotics in chicken meat [36]: identification of seven different chemical classes (amino glycosides, macrolides, lincosamides, sulfonamides, tetracyclines, quinolones and trimethoprim) has been carried out by liquid chromatography-mass spectrometry. Sample preparation including extraction with a mixture of acetonitrile: 2 % trichloroacetic acid (45:55, v/v). Samples were subjected to centrifugation and filtration was followed by on-line clean-up using turbulent flow chromatography. Larger number of samples can be analyzed using TCF per day than with a traditional clean-up technique (*e.g.* solid phase extraction).

Accurate testing of complicated food matrices [37]: Accurately monitoring contaminant levels in food and agricultural products is essential to assure the safety of the food supply

and to manage human health risks. It is well-known that the basic analytical requirements in food analysis are high-resolution, high-throughput, high-sensitivity detection and quantification of contaminants at or below the maximum residue limit or tolerance of the compound in a given food matrix. Liquid chromatography-mass spectrometry (LC/MS) as the central enabling technology has been recognized as an indispensable tool in the food safety and quality control fields. LC/MS provides high-speed, high resolution and high-sensitivity separation of various chemical compounds.

Veterinary analysis

Analysis of veterinary drug residues in honey [38]: Honey samples were diluted with an aqueous solution of Na_2EDTA (0.1 M). Then, they were injected into the chromatographic system including a turbulent flow chromatography column. Afterward, the analytes were transferred to an UHPLC analytical column, where they were determined by UHPLC-Orbitrap-MS. The developed method was applied in honey samples and it was fast and non-laborious.

Screening of veterinary drugs in milk [1]: Eight veterinary drugs, belonging to seven different classes were selected for this study. The screening method was then tested in the routine analysis of 12 raw milk samples.

Application in sample preparation for growth promoter and veterinary drug residue analysis [19]: the developments that have been made in multi-residue methods and particularly multi-class methods for residues of licensed animal health products.

Biochemical analysis

Analyses of hemodialyzates [39]: High turbulence liquid chromatography (HTLC, or turbulent flow online extraction) and tandem mass spectrometry (MS/MS) methods for the determination of sitagliptin in human urine and hemodialyzate were developed and validated to support clinical studies. A narrow bore large particle size reversed-phase column (Cyclone, 50 mm × 1.0 mm, 60 micron) and a BDS Hypersil C18 column (30 mm × 2.1 mm, 3 μ) were used as extraction and analytical columns, respectively.

Reduction of matrix effects in electrospray ionization mass spectrometry-based enzyme assays [40]: Turbulent flow chromatography (TFC) is presented as a means to reduce ion suppression in simultaneous multi analyte mass spectrometric bioassays. In this study, the effects of enzymes present in the sample on the signal response of five analytes were simultaneously investigated over a protein content range from 0 to 38 μg/mL by means of direct flow injection MS. As model enzymes, trypsin, thrombin and chymotrypsin were selected. Without employment of turbulent flow chromatography, both signal suppression and signal enhancement, depending on the nature of the analyte and the amount of matrix in the sample, were observed.

Analysis of biological samples with low-level metabolites [41]: The turbulent flow chromatography combined with column switching isocratic focusing was used to perform on-line sample preparation rat plasma for the identification of low-level metabolites. The concentration was achieved by focusing multiple injections, which were cleaned by a turbulent flow column, onto an analytical column prior to elution into the mass spectrometer. In addition, the first application of turbulent flow chromatography for on-line sample cleanup of neat bile samples is reported. The on-line cleanup and concentration method extracts and concentrates a sample 20-fold in 1h and is completely automated.

Solution-based ligand screening against multiple proteins [42]: used to remove the macromolecules followed by LC-MS analysis for identification and determination of the binding affinities. The technique can implement an ultra-fast isolation of protein/ligand complex with the retention time of a complex peak in about 5 s and on-line prepare the "clean" sample to be directly compatible with the LC-MS analysis. The improvement in performance of this 2D-TFC/LC-MS approach over the conventional approach has been demonstrated by determining affinity-selected ligands of the target proteins.

Protein-ligand screening/affinity ranking experiments [42]: Turbulent flow chromatography removes ligands, proteins and salts, followed by LC-MS analysis determination of the binding affinities. The improvement in performance of this 2D-TFC/LC-MS approach over the conventional approach has been demonstrated by determining affinity-selected ligands of the target proteins acetyl cholinesterase and butyryl cholinesterase from a small library with known binding affinities and a steroidal alkaloid library composed of structurally similar compounds.

Forensic applications

Application in drugs of abuse: Amphetamines: The use of turbulent flow chromatography (TurboFlow®) for online sample clean-up of urine samples for detection of amphetamines was developed to save time over conventional methods. Liquid liquid extractions are time consuming and cannot be fully automated to handle the high sample throughput required with this assay. Additionally, the current GC/MS methods also require labor intensive derivatization and sample clean-up steps. This Turbo Flow method provides better specificity for amphetamines through the duality that occurs in the Turbo Flow column. The patented size exclusion properties of Turbo Flow exclude the high molecular weight portion of the matrix, along with the salts, while the stationary phase coating retains the analyte(s) through reverse phase and anion exchange column chemistry. This results in an online separation prior to an analytical separation and introduction to the MS that is specific for amphetamines. This Turbo Flow method is able to analyze for amphetamines because the turbulent flow properties successfully separate matrix interferences from amphetamines, thus enabling analysis and detection.

Analysis of cortisol and a metabolite in urine [43]: Can be determined by using turbulent flow chromatography combines with LC-MS/MS technique.

Industrial applications

Determination of flavonoids and resveratrol in wine [44]: Flavonoids and resveratrol were determined by using turbulent-flow chromatography (TFC) on-line coupled to liquid chromatography mass spectrometry (LC-MS). 10 μL of sample was passed over a turbulent flow chromatography column, after which

the retained analytes were separated by reversed-phase LC and detected by negative ion mode atmospheric-pressure chemical ionization (APCI) MS. The method proved to be fast, non-laborious, robust and sensitive. The feasibility of the method was tested on several red, white and rose wines.

Analysis of endocrine disrupters and related compounds in sediments and sewage sludge [45]: the unequivocal identification and quantification of the most relevant environmental EDCs such as natural and synthetic estrogens and their conjugates, antimicrobials, parabens, bisphenol A (BPA), alkyl phenolic compounds, benzotriazoles and organophosphorus flame retardants, minimizing time of analysis and alleviating matrix effects. Applying this technique, after the extraction of the target compounds by pressurized liquid extraction (PLE), sediment and sewage sludge extracts were directly injected to the chromatographic system and the analytes were concentrated into the clean-up loading column. Using six-port switching system, the analytes were transferred to the analytical column for subsequent detection by MS-MS (QqQ). In order to optimize this multiplexing system, a comparative study employing six types of Turbo Flow TM columns, with different chemical modifications, was performed to achieve the maximum retention of analytes and best elimination of matrix components.

Conclusion

Based on this study, it is concluded that the turbulent flow chromatography is applicable for the analysis of a wide range of compounds. During the method development for determination of different compounds the sample preparation time can be substantially reduced compared to the existing sample preparation techniques. Coupling and on-line extraction approach allowing minimal sample preparation with a powerful analytical separation has to be the ultimate approach for bio-analysis. Compounds which are critical or tedious to separate using other sample preparation techniques like SPE and other extraction techniques can be easily separated by turbulent flow chromatography.

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REFERENCES

1. A.A.M. Stolker, R.J.B. Peters, R. Zuiderent, J.M. DiBussolo and C.P.B. Martins, *Anal. Bioanal. Chem.*, **397**, 2841 (2010); https://doi.org/10.1007/s00216-010-3660-5.
2. M.J. Berna, B.L. Ackermann and A.T. Murphy, *Anal. Chim. Acta*, **509**, 1 (2004); https://doi.org/10.1016/j.aca.2003.12.023.
3. L.H. Joseph and E. Tony, *LC GC N. Am.*, **30**, 200 (2012).
4. O. Reynolds, *Roy. Soc. Phil. Trans.*, **174**, 935 (1883); https://doi.org/10.1098/rstl.1883.0029.
5. A. Vintiloiu, W.M. Mullett, R. Papp, D. Lubda and E. Kwong, *J. Chromatogr. A*, **1082**, 150 (2005); https://doi.org/10.1016/j.chroma.2005.05.081.
6. J. Ayrton, G.J. Dear, W.J. Leavens, D.N. Mallett and R.S. Plumb, *J. Chromatogr. A*, **828**, 199 (1998); https://doi.org/10.1016/S0021-9673(98)00579-2.
7. T. Pretorius and T.W. Smuts, *Anal. Chem.*, **38**, 274 (1966); https://doi.org/10.1021/ac60234a030.
8. H.A. Kusch, J.M. Ottino and D.M. Shannon, *Ind. Eng. Chem. Res.*, **28**, 302 (1989); https://doi.org/10.1021/ie00087a009.
9. J. Baldyga, J.R. Bourne and S.J. Hearn, *Chem. Eng. Sci.*, **52**, 457 (1997); https://doi.org/10.1016/S0009-2509(96)00430-7.
10. M.C. Jones, R.D. Nassimbene, J.D. Wolfe and N.V. Frederick, *Chem. Eng. Sci.*, **51**, 1009 (1996); https://doi.org/10.1016/S0009-2509(96)80001-7.
11. G. Taylor, *Proc. R. Soc. Lond. A Math. Phys. Sci.*, **219**, 186 (1953); https://doi.org/10.1098/rspa.1953.0139.
12. A.A. Barresi, *Chem. Eng. Sci.*, **5**, 807 (1997); https://doi.org/10.1016/S0009-2509(97)00445-4.
13. R.B. Bird, W.E. Steward and E.N. Lightfoot, Transport Phenomena, Wiley, New York (1960).
14. E. Tony, in ed.: I.D. Wilson, Handbook of Analytical Separations: Turbulent Flow Chromatography in Bioanalysis, In: Bioanalytical Separations, vol. 4, Chap. 4, pp. 91-128 (2003).
15. M. Golay, in ed.: D. Desty, Gas Chromatography, Butterworth, London, pp. 35 (1958).
16. R. Aris, *Proc. R. Soc. Lond. A Math. Phys. Sci.*, **235**, 67 (1956); https://doi.org/10.1098/rspa.1956.0065.
17. R. Aris, *Proc. R. Soc. Lond. A Math. Phys. Sci.*, **252**, 538 (1959); https://doi.org/10.1098/rspa.1959.0171.
18. L.J. Tichacek, C.H. Barkelew and T. Baron, *AIChE J.*, **3**, 439 (1957); https://doi.org/10.1002/aic.690030404.
19. B. Kinsella, J. O'Mahony, E. Malone, M. Moloney, H. Cantwell, A. Furey and M. Danaher, *J. Chromatogr. A*, **1216**, 7977 (2009); https://doi.org/10.1016/j.chroma.2009.09.005.
20. R. Krebber, F.J. Hoffend and F. Ruttmann, *Anal. Chim. Acta*, **637**, 208 (2009); https://doi.org/10.1016/j.aca.2008.11.006.
21. J.M. Kevin, E. Chris and E. Francois, On-line Enrichment HTLC/MS/MS Assay for Multiple Classes of Antibiotics in Environmental Water Sources, Application Note 354, Thermo Fisher Scientific, Somerset, NJ, USA.
22. P.A. Segura, C. Gagnon and S. Sauvé, *Chromatographia*, **70**, 239 (2009); https://doi.org/10.1365/s10337-009-1097-9.
23. A.R. Breaud, R. Harlan, J.M. Di Bussolo, G.A. McMillin and W. Clarke, *Clin. Chim. Acta*, **411**, 825 (2010); https://doi.org/10.1016/j.cca.2010.02.067.
24. U. Ceglarek, J. Lembcke, G. Martin Fiedler, M. Werner, H. Witzigmann, J. Peter Hauss and J. Thiery, *Clin. Chim. Acta*, **346**, 181 (2004); https://doi.org/10.1016/j.cccn.2004.03.017.
25. F. Guo, Q. Liu, J. Shi, F. Wei and G. Jiang, *Talanta*, **119**, 396 (2014); https://doi.org/10.1016/j.talanta.2013.11.006.
26. A.G. Helfer, M.R. Meyer, J.A. Michely and H.H. Maurer, *J. Chromatogr. A*, **1325**, 92 (2014); https://doi.org/10.1016/j.chroma.2013.11.054.
27. A.K. Petrides, J. Moskowitz, K.L. Johnson-Davis, P.J. Jannetto, L.J. Langman, W. Clarke and M.A. Marzinke, *Clin. Biochem.*, **47**, 73 (2014); https://doi.org/10.1016/j.clinbiochem.2014.07.018.
28. R.C. Schofield, L.V. Ramanathan, K. Murata, M. Grace, M. Fleisher, M.S. Pessin and D.C. Carlow, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **1002**, 169 (2015); https://doi.org/10.1016/j.jchromb.2015.08.025.
29. G.-Z. Xin, J.-L. Zhou, L.-W. Qi, C.-Y. Li, P. Liu, H.-J. Li, X. Wen and P. Li, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **878**, 435 (2010); https://doi.org/10.1016/j.jchromb.2009.12.027.
30. Y. Xu, K.J. Willson, M.D.G. Anderson, D.G. Musson, C.M. Miller-Stein and E.J. Woolf, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **877**, 1634 (2009); https://doi.org/10.1016/j.jchromb.2009.04.006.
31. M. Llorca, F. Pérez, M. Farré, S. Agramunt, M. Kogevinas and D. Barceló, *Sci. Total Environ.*, **433**, 151 (2012); https://doi.org/10.1016/j.scitotenv.2012.05.080.
32. F. Perez, M. Llorca, M. Farré and D. Barceló, *Anal. Bioanal. Chem.*, **402**, 2369 (2012); https://doi.org/10.1007/s00216-011-5660-5.
33. B. Duretz, M. Manchon, J. Guitton, S. Cohen and C. Moreau, *Toxicol. Lett.*, **205(Suppl.)**, S60 (2011); https://doi.org/10.1016/j.toxlet.2011.05.314.
34. P. Mottier, Y.A. Hammel, E. Gremaud and P.A. Guy, *J. Agric. Food Chem.*, **56**, 35 (2008); https://doi.org/10.1021/jf072934d.

35. https://tools.thermofisher.com/content/sfs/brochures/TG-63551-Determination-Antibiotics-Milk-TG63551-E.pdf (22/06/2016).
36. K. Bousova, H. Senyuva and K. Mittendorf, *J. Chromatogr. A*, **1274**, 19 (2013); https://doi.org/10.1016/j.chroma.2012.11.067.
37. S. Yang, Accurate Testing of Complicated Food Matrices, Food Quality and Safety (2009).
38. M.M. Aguilera-Luiz, R. Romero-González, P. Plaza-Bolaños, J.L.M. Vidal and A. Garrido Frenich, *J. Agric. Food Chem.*, **61**, 829 (2013); https://doi.org/10.1021/jf3048498.
39. W. Zeng, D.G. Musson, A.L. Fisher, L. Chen, M.S. Schwartz, E.J. Woolf and A.Q. Wang, *J. Pharm. Biomed. Anal.*, **46**, 534 (2008); https://doi.org/10.1016/j.jpba.2007.11.003.
40. A. Liesener and U. Karst, *J. Sep. Sci.*, **28**, 1658 (2005); https://doi.org/10.1002/jssc.200500090.
41. J.L. Herman, *Rapid Commun. Mass Spectrom.*, **19**, 696 (2005); https://doi.org/10.1002/rcm.1843.
42. J.-L. Zhou, J.-J. An, P. Li, H.-J. Li, Y. Jiang and J.-F. Cheng, *J. Chromatogr. A*, **1216**, 2394 (2009); https://doi.org/10.1016/j.chroma.2009.01.010.
43. R. Grant, 15th IMSC, Barcelona, Spain (2000).
44. M.A. Presta, B. Bruyneel, R. Zanella, J. Kool, J.G. Krabbe and H. Lingeman, *Chromatographia*, **69**, 167 (2009); https://doi.org/10.1365/s10337-009-1132-x.
45. M.Gorga, S. Insa, M. Petrovic and D. Barceló, *J. Chromatogr. A*, **1352**, 29 (2014); https://doi.org/10.1016/j.chroma.2014.05.028.