Accelerating the Quality Control of Pharmaceuticals Using Monolithic Stationary Phases: A Review of Recent HPLC Applications

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Abstract

High-performance liquid chromatography (HPLC) is the predominant technique in the quality control of pharmaceutical formulations. HPLC offers highly sensitive and selective analytical methods with increased robustness and separation efficiency. The majority of HPLC-based methodologies are based on the usage of particulate-based columns and cannot be applied at elevated flow rates (> 2 mL/min) due to excessive back-pressure. Therefore, the typical duration of separation cycles is in the range of 5–30 min, making most assays time-consuming, especially when a lot of samples have to be analyzed in a minimum amount of time. Monolithic stationary phases for HPLC offer a very interesting alternative to conventional particulate-based columns. The size of the skeleton and the distribution of the pores of monolithic materials offer the possibility of developing efficient separation protocols at higher flow-rates due to the low pressure-drop across the column. The present review intends to cover the applications of monolithic based HPLC stationary phases in the quality control of pharmaceutical formulations, including identification of active pharmaceutical ingredient, assay, purity, dissolution, blending, and dosage uniformity, etc.

Introduction

Quality control (QC) is an important part of the pharmaceutical quality assurance of a drug before its commercialization. The aim of this process is to inspect and control the quality of the drug in terms of efficacy and safety (1,2). According to International Conference on Harmonization (ICH) guidelines, three analytical-based processes must be examined during the QC or stability testing of a drug, including (3): (*i*) assay [evaluation of the active pharmaceutical ingredient (API) concentration in drug-containing dosage form]; (*ii*) impurity determination (identification and quantification of API impurities); (*iii*) dissolution test (monitoring the rate of the release of API from oral pharmaceutical dosage).

Pharmacopoeias' guidelines (USP, EP, BP, JP) recommend extensive usage of high-performance liquid chromatography (HPLC) during validation of manufacturing processes and the QC of a drug. On this basis, automation and sampling throughput are therefore of great importance for the pharmaceutical industry because the analytical results determine the following steps in the production of the pharmaceutical products.

The column is the "heart" of an HPLC setup in which separation of compounds of interest is carried out. Until the last decade, typical commercially available HPLC columns consisted of a stainless steel tube uniformly packed with porous or non-porous micro-particles (4). The separation efficiency of such a column is mainly affected by the size and distribution of the particles. In contrary to particulate columns, monolithic columns are usually made of single rigid porous silica, which was initially called "silica rod" (5). Silica-based monoliths provide favorable properties for high-efficiency fast separations, such as low-pressure drop across the column, fast mass transfer kinetics, and a high binding capacity. The main characteristic of monolithic columns is that they allow higher flow-rates than particulate columns at reasonable back-pressures, expanding the possibilities of HPLC.

Monolithic stationary phase has become a "popular" separation media for HPLC among scientists, and day by day this technology is increasingly used in many HPLC applications (5). In the last few years, special attention has been given from many research groups on the usage of these materials in pharmaceutical industry. Particularly, the US Pharmacopoeia has introduced and adopted monolithic C_{18} columns, among the accepted L1 type material, for the QC of the drugs (6).

This review attempts to give an overview covering various aspects of monolithic columns in terms of synthesis, chromatographic characteristics, and their application on the QC of the drugs. Furthermore, special attention will be given to recently reported applications.

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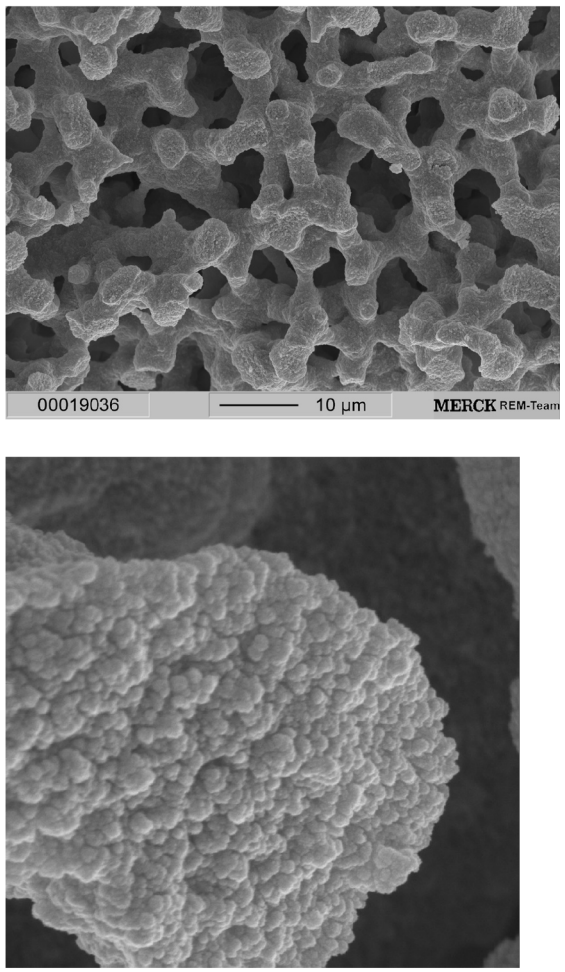

Figure 1. Scanning electron microscopy image of typical porous structure of monolithic silica-based column (A), mesopore of silica structure (B).

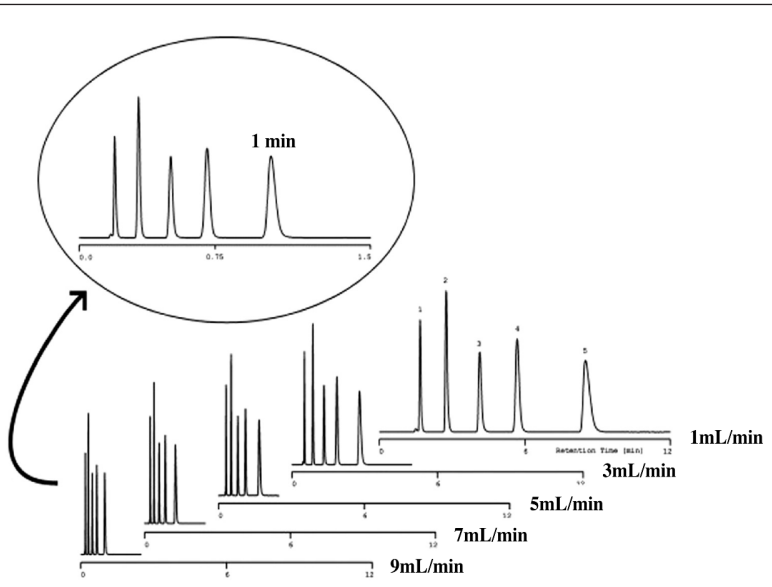

Figure 2. Separation of five β-blocking drugs using a monolithic silica column (Chromolith Performance RP-18e, 100 × 4.6 mm) at different flow rates (5).

Monolithic Columns

Synthesis of monolithic columns

Historically, Nakanishi et al. (7) synthesized the first porous silica monolith material based on the simultaneous hydrolysis and condensation of tetramethoxysilane (TMOS) in the presence of polyethylene oxide under acidic conditions. The monolithic material obtained was like a "chalk" consisting of macro- and meso-pores. Following this, the research group of Tanaka (8) first investigated the chromatographic properties of monolithic material in terms of separation performance and analysis time. The rod-type column had a length of approximately 80 mm and 7 mm internal diameter. Merck company has recently commercialized this column (Chromolith), which is suitable for HPLC applications in many analytical fields (9). Up to now, several research works (10–14) and reviews (15–18) have been published regarding the preparation of monoliths and their applications.

The general procedure for synthesis of silica-based monolithic columns is mainly based on the in situ polymerization of organic monomers. Generally, two different reaction types take place simultaneously (hydrolysis and condensation), converting alkoxysilane to silica gel oligomers and polymers in the presence of a porogen (e.g., polyethylene glycol) (14, 19). Typical alkoxysilanes used are TMOS or tetraethoxysilane. During the sol-gel process, a network structure of silica gel skeleton with macropores is formed while the skeleton diameter and permeability can be controlled, altering the concentration of porogen in the reactant mixture. Therefore, the macroporous (~ 2 µm) network structure of silica gel skeletons contains mesopores (~ 13 nm), which are subsequently formed by treatment of the polymer under alkaline medium (20) (Figure 1). Finally, the material is dried and the surface functionalized with ligand-giving hydrophobic, ion-exchange, or affinity properties. From a mechanical point of view, the produced monolith is fitted into a fritless polytetrafluoroethylene, polyaryletheretherketone, or capillary tube in a way so that the mobile phase passes through the material. Other procedures for the synthesis of silica-based monoliths are described in depth in an interesting survey article by Guiochon (18).

On the other hand, polymeric monolithic columns can be described as an assembly of fused micro-globules with graded densities (21). This type of monolith is characterized by smaller pores; namely, meso- and micropores, while the specific surface area is much smaller than that of monolithic silica. The polymeric monoliths are considered to be advantageous over the silica-based monolith because of the specific groups (e.g., C_{18}, C_8, SO_3^-) that can be functionalized at the material by the addition of appropriate comonomers in the reactant mixture. Further, polymeric monoliths are "fitted" in a variety of column types as thin membranes, disks,

capillary columns, and large bore preparative assemblies (22–24).

The first polymeric monolith was fabricated by Hjerten et al. (25–27), where a compressed polyacrylamide gel was prepared after complex and laborious process. Considerable research efforts were made by Hjerten and co-workers to simplify their methodology. Later, Svec and Frechet (28,29) first reported a remarkably simpler procedure for the preparation of polymeric monoliths in capillary tubes. Nowadays, these monolithic materials are prepared from synthetic polymers; namely, polymethacrylate, polyacrylamide, and polystyrene (30–32), and natural polymers such as agarose and cellulose (33). Many companies such as Merck, BIA Separations, LC Packings, etc., commercialized these materials in disk, rod, or tube format.

The main approach for polymeric monolithic material manufacturing consists of three steps: (*i*) treatment (e.g., silinization) of the column tube wall in which the polymer will be synthesized adheres strongly to the internal surface of the column. This procedure prevents the mobile phase from flowing between the polymeric monolith and the wall of the column; (*ii*) polymerization of an appropriate reaction mixture under controlled temperature. The reagents used depend on the desired properties (hydrophilic or hydrophobic) of the monolith. In both of the cases, a porogen (e.g., dextran, dodecanol) is necessary to be added for the creation of pores; (*iii*) chemical modification of the polymer surface in order to give the desired functional group (34,35).

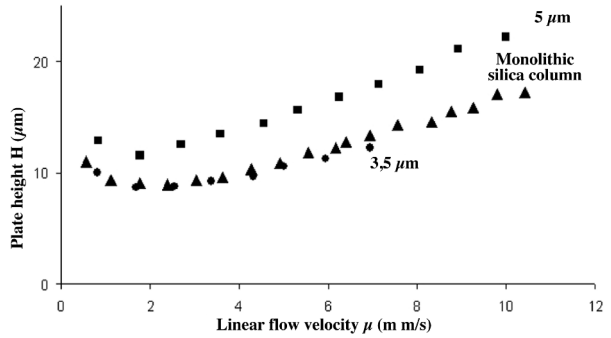

Figure 3. Van Deemter curves for the monolithic and particulate columns with silica particles of different diameters (5).

Table I. Chromatographic Parameters of Monolithic Silica Column (Chromolith® Performance, RP 18e) and Microparticulate Silica Columns (C_{18} Reversed phase) (21).

Chromatographic parameters	Monolithic column*	Micro-particulate column
Column hardware	PEEK	usually stainless steel
Column dimensions (length/i.d.) (mm)	100 / 4.6	100–250/4–4.6
Column pressure drop (bar) (at 1 mL/min)	< 20	40–150
Volume flow rate range (mL/min)	0.01-10	0.01–5
Plates numbers values† (N/m)	~80,000	30,000–110,000

* Chromolith Performance (RP 18e) (Merck).
† Values are given for a neutral compound.

Chromatographic properties of monolithic columns—comparison with particulate columns

The most important features of monolithic columns are their high porosity resulting from the network of macropores and the structure of the stationary phase skeleton. These two structural characteristics permit the combination of a low hydraulic resistance of the column to the mobile phase and an enhancement of the mass transfer rate of the analyte molecules through the material (21). Another characteristic is that the dimensions of the skeleton and consequently the size of the pores can be adjusted by controlling the concentration ratio of the reactants in the starting mixture. Thus, large through-pore size/skeleton size ratios and high porosities can be achieved, providing high permeability and a large number of theoretical plates per unit pressure drop (15,21).

Comparison of monolithic and particulate columns reveals that the mass transfer process is significantly higher in the case of monolithic columns. On this basis, high permeability permits fast separations, and consequently, high sampling throughput. For instance, Figure 2 illustrates that the separation of beta-blocking drugs was achieved in less than 1 min when a flow rate of 9 mL/min was employed (5). Experiments on this topic showed that the van Deemter plot appeared to be very "shallow", which means that fast separations can be carried out without loss of efficiency (5,36) (Figure 3). However, the separation efficiency achieved using monolithic silica columns is generally lower than those of particulate columns (packed with 4 or 5 µm particles) because its eddy diffusion value is higher than particulate ones. In terms of permeability, the monolithic columns have similar permeability with particulate columns packed with 10- or 12-µm particles. A comparison of chromatographic parameters of monolithic and particulate columns is tabulated on Table I (21).

Finally, another beneficial point of the monolithic column over particulate-based columns is that the preparation of narrow-bore monolithic columns is easier than narrow-bore particulate columns, and the reproducibility of the former is better. This explains why the usage of narrow-bore packed columns has been practically abandoned (18).

Applications

Drug assay

An assay of finished pharmaceutical products (e.g., suspension, tablet, capsule) is a critical part of the quality assurance process. On this basis, a valid analytical procedure is required to provide reliable data regarding to the drug concentration in its formulation. The analytical characteristics of monolithic-based HPLC methods for the assay of pharmaceutical formulations can be found in Table II.

A series of studies has been published by Aboul-Enein dealing with the determination of various APIs (clopidogrel, haloperidol, vardenafil, tadalafil, sildenafil, and lamivudine) in their respective pharmaceutical formulations (37–42). Different mobile phase compositions, including mixtures of acetonitrile with water or phosphate buffer, were employed for the fast and reliable determination of each drug in pharmaceuticals. Isocratic elution was performed using a flow-rate of 1 (38), 2 (39,41,42), 4 (37), and 5 mL/min (40), resulting in analysis times in the range of 1 to 10 min depending on the analyte. Each proposed method was validated in terms of linearity, precision and accuracy, and limits of detection and quantification (LOD and LOQ). A comparison of monolithic column performance with a particle-based column was carried out for the determination of vardenafil (39). Specifically, the retention time of the analyte was decreased at 2 min when a monolithic column (100 mm × 4.6 mm i.d.) was utilized at a flow rate of 2 mL/min. Furthermore, the analysis of vardenafil was sped up with a 10% increase in the percentage of acetonitrile in the mobile phase.

An interesting chromatographic approach with significant potential has been reported by Schneider et al. for the quantitation of amphetamines in illicit ecstasy tablets (43). Triethylamine was used as ion-pair reagent to achieve separation between analytes of interest. The flow-rate pumping through a C_{18} monolithic column (Chromolith SpeedRod RP-18e column, 50 mm × 4.6 mm i.d.) was examined in the range of 1.5 to 4 mL/min, resulting in an analysis time of 3.5 to 11 min. Three years later, Fadden et al. worked further on this topic, investigating the separation of principal amphetamines using three C_{18} monolithic columns of various lengths (25, 50, and 100 mm) (44). In terms of efficiency, monolithic columns were comparable with particle-based columns, especially when high flow-rates are applied. Specifically, the same efficiency was observed for the SpeedRod monolithic column (50 mm × 4.6 mm i.d.) at 7 mL/min with the particulate Waters XTerra RP18 (250 mm × 2.1 mm i.d.) at 3 mL/min. Successful separation of six amphetamines was achieved at a flow-rate of 4.5 mL/min with a reasonable analysis time. The LODs of the analytes ranged from 0.2 to 3 µg/mL. The proposed method was applied for the chemical profiling of ecstasy tablets.

Solid-phase extraction (SPE) was employed by Hashem et al. as a sample pretreatment step prior to the analysis of three corticosteroids by HPLC (45). The SPE procedure included acti-

Table II. Analytical Figure of Merits of Drug HPLC Assays Using Monolithic Columns

API	Mobile phase	Column dimension (length × i.d.) (mm)	Flow-rate (mL/min)	Wavelength (mn)	Linear range (µg/mL)	LOD (µg/mL)	Ref.
Clopidogrel	acetonitrile–phosphate buffer (50:50, v/v, pH 3.0) (IE*)	100 × 4.6	4.0	235	1–40	0.97	(37)
Haloperidol	acetonitrile–phosphate buffer (70:30, v/v, pH 3.0) (IE)	100 × 4.6	1.0	230	0.1–10	0.1	(38)
Vardenafil	acetonitrile–phosphate buffer (30:70, v/v, pH 3.0) (IE)	100 × 4.6	2.0	230	10–1000	0.31	(39)
Tadalafil	acetonitrile–phosphate buffer (80:20, v/v, pH 3.0) (IE)	100 × 4.6	5.0	230	0.1–5	0.1	(40)
Sildenafil	acetonitrile–water (60:40, v/v) (IE)	100 × 4.6	2.0	292	0.05–3	0.025	(41)
Lamivudine	acetonitrile–water (65:35, v/v) (IE)	100 × 4.6	2.0	285	0.025–0.8	0.0125	(42)
Amphetamines	acetonitrile–phosphate buffer + 0.1% triethylamine (GE†)	50 × 4.6	1.5	210, 285	100–500 67.2–336	4.05–15.13	(43)
Amphetamines	acetonitrile–phosphate buffer (3:97, v/v) (IE)	25,50,100 × 4.6	3.0	200	–	0.2–3	(44)
Corticosteroids	methanol–water (50:50, v/v) (IE)	100 × 4.6	1.0	254	1–10	0.25–0.5	(45)
Cephalosporins	methanol–acetate buffer (10:90, v/v) (IE)	50 × 4.6	1.8	265	–	0.001–0.025	(46)
Acyclovir	acetic acid (0.2%) (IE)	100 × 4.6	2.0-5.0 (FG‡)	254	5–120	0.05	(47)
Nicotine	3.3% w/w SDS, 6.6% w/w butanol, 0.8% w/w	100 × 4.6	4.0	220, 254	4.3–87	0.004	(48)
Naproxen	octane, 0.05% v/v TFA in water (IE)				108–538		

* IE: isocratic elution.
† GE: gradient elution.
‡ FG: flow gradient.

vation and cleaning of the sorbent with methanol and water, followed by the loading of sample. A series of mixtures of methanol–water were passed through the SPE cartridge to remove the potential interferences while the analytes were eluted by washing with pure methanol. The method was appropriately validated in terms of linearity, precision, accuracy, LOD/LOQ, and robustness. Fast determination of three compounds was carried out in less than 6 min.

A 100 mm long reversed-phase monolithic column has been utilized by the research group of Papadoyannis for the separation and determination of four cephalosporins in their respective pharmaceutical formulations (46). Adequate resolution (ranging from 0.8 to 1.5) was achieved in less than 4 min at a flow-rate of 1.8 mL/min. An eluent consisting of acetate buffer and methanol in a ratio of 90:10 (v/v) was employed for analyte separation. The LODs varied from 1 to 25 ng/mL. The developed assay applied for the determination of four cephalosporins in pharmaceuticals (capsules, oral suspension, and sterile powder for injection).

Tzanavaras et al. used a typical HPLC setup with a monolithic column (100 mm × 4.6 mm i.d.) to study three different extraction protocols of acyclovir from pharmaceutical creams (47). Ultrasonication with and without heat and magnetic stirring were evaluated and compared in terms of rapidity, precision, and extraction efficiency. A stepwise flow gradient was followed to ensure rapid elution of acyclovir molecule at less than 3.5 min. Validation of calibration curve was carried out by the response factor test.

A recent, more sophisticated method for the determination of nicotine and naproxen in lozenges and tablets involved an oil-in-water microemulsion as eluent (48). Microemulsion consisted of sodium dodecylsulphate in water mixed with trifluoroacetic acid. Then, octane and butan-1-ol were added and the solution was sonicated to form an optically transparent microemulsion. The separation capability of this eluent was evaluated for the separation of parabens under isocratic elution using a flow-rate of 4 mL/min. The use of oil-in-water microemulsions with monolith columns (Chromolith Performance RP-18e 100 × 4.6 mm i.d.) has excellent potential for high-speed quantitative analysis.

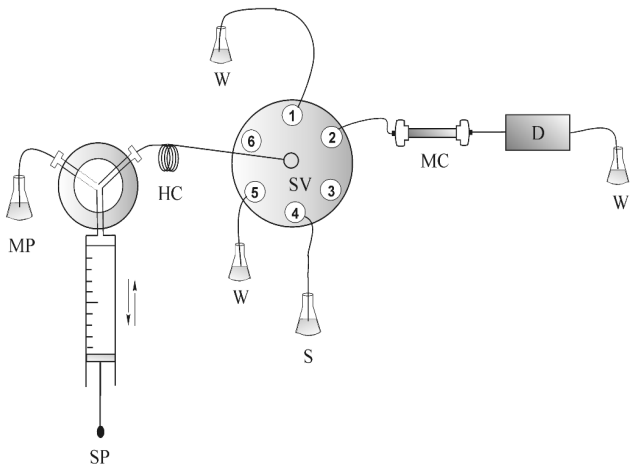

Figure 4. Typical configuration of the SIC setup: MP, mobile phase; SP, syringe pump; HC, holding coil; SV, selection valve; MC, monolithic column; S, sample; W, waste; D, detector.

The potential of monolithic columns is pointed out to a great extent when sequential injection setup is used instead of typical high-pressure liquid chromatographic configurations. This separation technique is called sequential injection chromatography (SIC), and it was developed in 2003 by Huclova et al. (49). Recently, a detailed review has been published dealing with the applications of SIC in pharmaceutical analysis (50). A typical SIC configuration consists of an integrated sequential injection analysis setup where a short monolithic column is positioned in a flow line of a multi-position valve before the detector (Figure 4). Looking at the operational procedure, the well-defined sample zone is injected in the system and propelled towards the column for separation. Then the mobile phase, acting as carrier, is employed to elute each compound from the monolithic column at relatively high flow-rate (51). An alternative low-pressure liquid chromatographic method has been published by Obando et al. for the quantitation of hydrochlorothiazide and losartan in tablets (52). The system comprised a multisyringe module instead of high-pressure pump connected with a short monolithic column via solenoids valves. The developed assay was based on two basic principles. Firstly, one of the two syringes was filled with mobile phase and at the same time the loop was loaded with sample. Next, changing the positions of the solenoid valves and the mobile phase passed through the loop and the sample was driven towards to the monolithic column. Separation of two analytes was carried out performing simple isocratic elution. The results obtained were in good agreement with those from a conventional HPLC method.

Dissolution test

The dissolution test as defined in the United States Pharmacopoeia is a method for evaluating physiological availability that depends upon having the drug in a dissolved state (53). In other words, it is a monitoring of the release rate of a drug substance when a pharmaceutical dosage formulation is administrated orally (54). The effectiveness of such a process relies on the drug dissolving in the fluids of the gastrointestinal tract prior to absorption into the circulation. Nevertheless, dissolution data exhibit useful information concerning to the bioavailability of a drug and also in product uniformity assessment, in terms of site and scale of manufacture, and manufacturing process and equipment (55–58).

In practice, the construction of the dissolution profile of a drug requires HPLC analysis of a significant amount of samples in order to determine the concentration of API at defined time intervals. On this basis, rapidity and efficient separation are the main goals of an HPLC method used for this purpose. This demand is fulfilled employing a monolithic column instead of a particle-based column; hence, it provides high sampling throughput due to the short analysis time.

A detailed study on the dissolution test of selegiline has been reported by Tzanavaras et al. (59) using a commercially available HPLC configuration coupled to a Chromolith RP-18e monolithic column (100 mm × 4.6 mm i.d.). A mixture of

phosphate buffer and acetonitrile was employed as eluent and pumped through the column at a flow-rate of 3 mL/min. The proposed method enabled the determination of the analyte in less than 1 min, producing a sampling rate of 60 samples per hour. The calibration curve was found to be linear in the range of 0.5–15 mg/L of the drug, while the precision and the accuracy of the method ranged between 0.75% to 2.3% and 99.0% to 101.6%, respectively. The LOD and LOQ achieved were approximately 30 and 100 µg/L. Selectivity and ruggedness were also investigated to prove the reliability of the proposed method. Careful investigation and optimization of the instrumental parameters of the dissolution test were carried out; namely, dissolution medium, basket and paddle agitation, and rotation speed.

A shorter monolithic column (Chromolith, 50 × 4.6 mm i.d.) was utilized by the same author for the dissolution stability control of nimesulide-containing pharmaceutical formulations (60). The selectivity of the developed HPLC assay was evaluated, separating the analyte against its potential impurities. Furthermore, the analysis time was investigated by changing the flow-rate of the mobile phase in the range of 2 to 5 mL/min. Successful determination of the analyte was achieved in less than 1 min using isocratic elution at 4 mL/min. The developed HPLC assay was validated in terms of linearity, LOD, LOQ, within-day, and day-to-day precision and accuracy. The effects of accelerated (for 6 months) and long-term (18 months) stability on the dissolution of nimesulide tablets were the main purpose of this contribution. According to the authors, the percent dissolution values were higher than the established specifications of the product (70% at 15 min and 85% at 30 min).

Stability test

Stability testing is a major task of pharmaceutical industries, during the development of new APIs and formulations, or routine production of commercially available products. The stability test is accomplished under recommended storage conditions (humidity, long-term stability, temperature, etc.) of the API and/or the finished product in order to ensure that the pharmaceutical formulation is safe and effective during the established self-life period (61). One of the critical parameters of stability testing is the investigation of incompatibilities during pre-formulation studies. These processes aim to identify the weak points of the API, in order to avoid vulnerable conditions in the formulation. The stability tests are performed under defined conditions with respect to temperature and humidity. In the case of accelerated tests, stability is evaluated in a 6-month period under a temperature of 40ºC and a relative humidity of 75%. Long-term stability involves storage of the formulation under normal conditions (25ºC, 60% relative humidity) for a period of 36 or 48 months. In these studies, HPLC comprised with monolithic columns is the predominant powerful technique capable of quantifying the API content rapidly without disturbance from potential interfering degradation products.

As mentioned in the previous section, Tzanavaras et al. (60) utilized a monolithic HPLC column to investigate the stability of nimesulide drug in its pharmaceutical formulations. Calibration curve of the analyte was evaluated in the range of 50–150 mg/L, while the accuracy of the proposed method was tested by analyzing placebo samples spiked with known amounts of nimesulide. The proposed assay provides a rapid determination of the analyte in samples after accelerated and long-term storage conditions.

An ion-pair liquid chromatographic approach was developed by Vasbinder et al. and applied to the separation and determination of *p*-aminosalicylic acid and its by-product; namely, *m*-aminophenol in pellets (62). Tetrabutyl-ammonium hydrogen sulphate was employed as the ion-pair reagent on a C_{18} monolithic column (50 mm × 4.6 mm i.d.) in order to improve the resolution and peaks' shapes. A flow gradient program (from 1 to 6 mL/min) was adopted, speeding up the separation and quantitation of compounds. The analytes were monitored using UV detector at 233 nm. Critical comparison and discussion of the chromatographic characteristics of monolithic columns with a particular-based column (RP-18 5 µm, 125 mm × 4 mm i.d.) having a similar length was also carried out. The experiments have shown that using the monolithic column at a flow-rate of 1 mL/min resulted in an analysis time of 16 min, while a 5-fold reduction was achieved employing a flow gradient program up to 6 mL/min. Similar relative retention times were recorded with both columns, while the LOQs were lower in the case of monolithic columns. The method was validated in terms of linearity, LOD/LOQ, precision and accuracy, selectivity, and robustness. The assay was applied for the stability testing of *p*-aminosalicylic acid in its pharmaceutical samples after 1, 3, and 6 months of storage.

A detailed study has been published by Pavli et al. for the separation and determination of bacitracin and bacitracin zinc from a complex mixture of several polypeptides (63). The separation of analytes was thoroughly investigated using isocratic or gradient elution on monolithic (Chromolith RP-18e, 100mm × 4.6mm i.d.) and particulate columns (Synergi Hydro-RP C_{18} and Hypersil BDS-C_{18} – 250 mm × 4.6mm i.d. each). Both isocratic and gradient methods were validated and critically compared in terms of analysis time, linearity, precision and accuracy, and LOD and LOQ. Under gradient elution, the use of the monolithic column was found to be advantageous over the particulate column because only 6 min were required to separate the analytes instead of 46 min. The method could be used for the routine QC of the bacitracin-containing formulations and in stability studies.

Drug impurity determination

In the last few years, special attention has been given by pharmaceutical industries to the identification and quantitation of any impurities presented in drug-containing pharmaceuticals. This has become quite evident by the review articles on this topic (64–69). The impurities in drugs often possess unwanted pharmacological or even toxicological effects in humans or animals. According to ICH guidelines (ICH Q3A) and US Pharmacopoeia, drug substance impurities can be classified in three main categories (70): (*i*) inorganic impurities (catalyst, salts, residual metals, etc.); (*ii*) organic impurities (by-products, intermediates, degradation products, etc.); (*iii*)

residual solvents (used for the preparation of drug solutions or suspensions during drug synthesis or product manufacturing).

The main feature of analytical techniques intended for this purpose should be the ability to separate the compounds of interest. Among analytical techniques, HPLC, capillary electrophoresis, and gas chromatography are commonly used for impurity identification and quantification.

Deeb et al. reported a specific method for the determination of glimepiride, glibenclamide, and its related compounds (71). Fast separation of four compounds (~ 80 s) was performed, exploiting a flow gradient from 5.0 to 9.9 mL/min at 1.3 min without sacrificing resolution or run-to-run reproducibility. Chromatographic parameters (e.g., percentage of organic modifier, column temperature, etc.) affecting the separation were examined and optimized. The efficiency of the Chromolith Performance monolithic column (RP-18e, 100 mm × 4.6 mm) was appraised constructing van Deemter plots for each analyte, employing a mixture of phosphate buffer and acetonitrile (55:45 v/v) as eluent.

An interesting method has been proposed by Tzanavaras et al. for the determination of acyclovir and its major impurity, guanine, in pharmaceutical raw materials and creams (72). Sample preparation of the cream-based matrix employed the dilution of sample in alkaline medium, stirring, ultrasonication, and finally, filtration prior to the analysis. A simple flow gradient protocol was followed to accelerate the elution of acyclovir while successful separation of the analytes was obtained in less than 3 min. The linearity of the assay was 80–120 µg/mL and 0.1–1.0 µg/mL for the acyclovir and guanine, respectively. The LODs achieved were 50 and 20 ng/mL, respectively. The method was applied to the QC of raw material and pharmaceutical cream after accelerated and long-term stability.

A UV detector (at 254 nm) coupled to the HPLC system has been utilized by Liu et al. for the quantification of rifampicin and its impurities (73) in pharmaceuticals. A mixture of citric acid, phosphate, methanol, and acetonitrile was pumped through a Chromolith Performance monolithic column (RP-18e, 100mm × 4.6 mm i.d.) at a constant flow-rate of 2 mL/min. The proposed method was validated in terms of linearity, precision, and accuracy. The chromatographic performance of the monolithic column was comparatively studied against a C_{18} particulate column (AT. Chrom C_{18}, 150 mm × 4.6 mm i.d.). A faster separation of rifampicin and its four impurities was achieved using the monolithic column (< 10 min), then the particulate one (~ 56 min) (Figure 5).

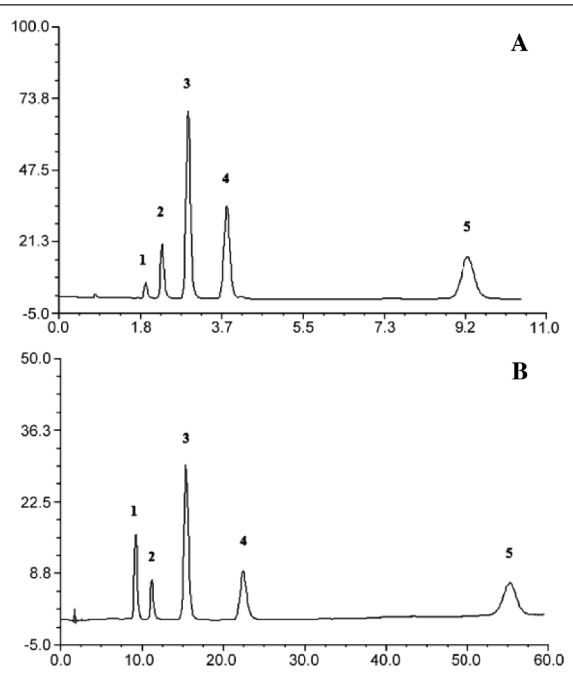

Figure 5. Separation of rifampicin and its four impurities on a monolithic column (A) and a conventional column (B) (73).

Impurity profiling of a Taxol analog (BMS-275183-01) was investigated by Rocheleau et al. using a monolithic column (Chromolith 100 mm × 4.6 mm i.d.) incorporated into an HPLC setup (74). The separation of the impurities was examined, manipulating different types of conventional silica-based HPLC columns. The effect of mobile phase composition on the peak resolution was also investigated. Satisfactory linearity in the range of 25–600 µg/mL was achieved, while the repeatability of the method was 0.4%. Additional validation parameters included accuracy and precision. The experimental findings reveal that the performance of the monolithic column is comparable to the silica particle-based column (YMC ODS-AQ, 250 mm × 4.6 mm i.d.).

A similar comparative study has been exploited by Novakova et al. using ultra-performance liquid chromatography and HPLC with a monolithic column (100 mm × 4.6 mm i.d.) to determine diclofenac and its degradation compound (75). Chromatographic parameters; such as, resolution, plate numbers, asymmetry, and repeatability of the retention times were studied using a series of five different analytical columns (Zorbax Eclipse XDB-C18 75 × 4.6 mm; Zorbax Eclipse SB-C18 50 × 4.6 mm; Purospher RP 18e 125 × 4.0 mm). In terms of analysis time, the monolithic column was found to be superior (~ 1.8 min) against other columns tested.

An Onyx (Phenomenex, C_{18}, 100 mm × 4.6 mm i.d.) monolithic column coupled with an HPLC system was utilized by the research group of Carrier for the determination of indazole, its impurity (4-hydroxybenzoic acid), and commonly used preservatives (parabens) (76). A fast gradient elution program was followed at a flow-rate of 5.8 mL/min. The wavelength of the UV detector was set at 254 nm. The method was found to be precise and accurate, while the linearity for the determination of 4-hydroxybenzoic acid ranged between 0.15 to 0.44 µg/mL. A robustness test was also carried out.

Finally, Schmidt reported a fast HPLC method for the QC of *Harpagophytum procumbens* in its phytopharmaceutical preparations (77). The main target of this approach was the method transfer from a conventional particulate column (Hypersil ODS, 125 mm × 4 mm i.d.) to a monolithic column (Chromolith Performance RP-18e 100 mm × 4.6 mm i.d.). An 85% reduction in analysis time was accomplished using a monolithic column at 5 mL/min, even if two columns are connected in series. From a statistical point of view, no significant differences in the results obtained were found using both columns. The main drawback of the proposed assay is that the cost of two monolithic columns is approximately three times higher than a particulate column.

Practically, this shortcoming can be eliminated by taking into account that in pharmaceutical industries, the speed of analysis is crucial and cost-effective.

Conclusions

The monolithic columns exhibit a unique characteristic: high permeability. This feature yields the ability to apply relative high flow-rates (up to 10 mL/min) through the column, reducing the analysis time. The majority of the researchers that have worked on this topic state that monolithic and conventional particulate columns are comparable with respect to performance, selectivity, and reproducibility.

Monolithic media can be considered as one of the best and most feasible solutions for the pharmaceutical industry for QC purposes. Critical parameters (assay, impurity determination, dissolution tests) affecting the quality assurance of the drug can be effectively accelerated using a monolithic column instead of a conventional column. This is the reason that recent editions of pharmacopoeias have introduced the concept of monolithic columns in routine analysis.

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