Automated Quality Control of Pharmaceuticals using Sequential Injection Chromatography (SIC)

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The present piece is my third Editorial contribution to the international journal *Pharmaceutica Analytica Acta*. The first Editorial (<http://dx.doi.org/10.4172/2153-2435.1000105e>) discussed some potential of automated flow injection techniques in the field of pharmaceutical analysis, focusing on chemiluminescence detection and flow optosensors [1]. The topic of the second Editorial (<http://dx.doi.org/10.4172/2153-2435.1000e114>) was more specialized discussing the applications of Sequential Injection Analysis (SI) to drug dissolution studies [2]. This third Editorial is also focused on automated flow techniques and will present theoretical and practical aspects of one of the newest members of the family of automated flow injection techniques, namely Sequential Injection Chromatography (SIC).

Despite the hundreds publications using Flow (FI) and Sequential Injection Analysis (SI) reporting the automated analysis of pharmaceuticals, the majority of the methods can be characterized as “single analyte assays” [3]. This is an important drawback of automated low pressure flow techniques, although there are various attempts towards this direction. These reports were in many cases ingenious, but in general were rather complicated or required strictly controlled conditions and were typically based on kinetic discrimination [4], on-line sample splitting [5] or usage of different detection systems [6]. On the other hand, simultaneous multi-component analysis is critical in many aspects of the quality control of pharmaceuticals. For example there are numerous two- or three-active component formulations (e.g. combinations of antihypertensive and diuretic ingredients). Additionally, purity control and stability testing of pharmaceuticals require per se the usage of analytical techniques with separation capabilities.

The breakthrough in multi-species determinations by FI or SI was made very recently by the incorporation of short monolithic columns to low pressure continuous flow manifolds, introducing the concept of Sequential Injection Chromatography (SIC) [7]. Reversed phase monolithic columns of various dimensions suitable for HPLC applications in many analytical fields were commercialized by Merck in 2000 [8] and were followed by other manufacturers (e.g. Phenomenex). The structural properties of monolithic stationary phases allow efficient separations typically comparable to 3-5 μm particulate columns at usually ten-times lower back pressures. This can be achieved by controlling independently the permeability (μm -sized throughpores) and efficiency of the analytical columns (nm-sized mesopores) [9] (Figure 1).

In a typical SIC configuration (Figure 2) a short monolithic column (5-50 mm long $\times$ 4.6 mm i.d.) is placed in the flow line between the multiposition valve and the detector. Depending on the used pump (syringe pump or peristaltic) the mobile phase can either flow continuously through the holding coil to the column (peristaltic pump) or can be aspirated prior to every run (syringe pump). Defined volumes of the samples at the microliters level are injected in the usual way in

the holding coil followed by propulsion to the column by selecting the appropriate port of the multiposition valve and by reversing the flow direction of the pump. Due to the pressure limitations of conventional SI setups, mobile phase flow rates are typically lower than 1.0 mL min⁻¹. Fast separations and short analysis cycles are based on the short length of the monolithic columns, while chromatographic efficiency depends on the selection of the mobile phase and on the properties of the analytes. The interested reader can find useful information on SIC in two excellent review articles that have been published recently [10,11].

Some representative pharmaceutical applications of SIC can be found in Table 1 [7,12-22]. Looking at the methods and the analytical conditions included in Table 1 it is evident that most of the so far reported and published protocols involve separation of simple two/three component mixtures employing isocratic elution and universal UV detection. To my opinion the potentials of SIC and related techniques can be demonstrated to the scientific community by mainly two ways: (i) by developing SIC methods with gradient elution that will expand the separation capabilities of the technique to more complicated mixtures, and (ii) by exploiting and demonstrating the automated sample treatment potentials of SI combined to monolithic-based separations. Interesting work towards these directions has been carried out by some research groups. For example, Zacharis et al. [23] applied on-line filtration and dilution protocols prior to SIC separation that allowed direct analysis of samples from stress degradation studies of acyclovir (Figure 3) [23]. In 2010 Chocholous and co-workers proposed a two-column SIC setup that enables the separation of analytes with different polarities with isocratic elution [24], while the same research group one year later exploited the potentials of gradient formation in the holding coil by reproducible mixing of two mobile phases [25].

The common denominator of all the SIC procedures mentioned and discussed so far in this editorial was the usage of monolithic stationary phases. Very recently Chocholous et al. [19] reported, for the first time, an interesting alternative that could expand the potentials of SIC [26]. This alternative was the incorporation of short HPLC columns based on the novel “fused core” or “core shell” technology. This technology is

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Analytes	Analytical Column	Detection	Mobile Phase	Flow rate (mL min ⁻¹)	Reference
Salicylic acid/Methyl salicylate	Chromolith® SpeedRod (50 × 4.6 mm i.d.)	UV at 240 nm	ACN-water (36/65) pH=2.5	0.6	[7]
Methylparaben/ Propylparaben/ triamcinolone acetonide	Chromolith® FlashRod (25 × 4.6 mm i.d.)	UV at 243 nm	ACN-MeOH-water (36/65) +0.05% nonylamine, pH=2.5	0.6	[12]
Methylparaben/ Propylparaben/ diclofenac	Chromolith® FlashRod (25 × 4.6 mm i.d.)	UV at 275 nm	ACN-water (40/70) +0.05% triethylamine, pH=2.5	0.48-1.2	[13]
Ambroxol hydrochloride / deoxycycline	Chromolith® FlashRod (25 × 4.6 mm i.d.)	UV at 213 nm	ACN-water (20/90), pH=2.5	0.48	[14]
Ambroxol hydrochloride / methylparaben / benzoic acid	Chromolith® FlashRod (25 × 4.6 mm i.d.)	UV at 245 nm	ACN-THF-0.05 M CH ₃ COOH (10/10/90), pH=3.75	0.48	[15]
Lidocaine/prilocaine	Chromolith® FlashRod (25 × 4.6 mm i.d.)	UV at 212 nm	ACN-0.05 M Phosphate (40/80) + 0.01% triethylamine	0.6	[16]
Naphazoline nitrate / methylparaben	Chromolith® FlashRod (25 × 4.6 mm i.d.)	UV at 220 nm and 256 nm	MeOH-water (40/65), pH=5.2	0.9	[17]
Betamethasone / Chloramphenicol	Chromolith® FlashRod (25 × 4.6 mm i.d.)	UV at 241 nm and 278 nm	ACN-water (30/80)	0.48	[18]
Triamcinolone acetonide / Salicylic acid	Onyx® C18 (50 × 4.6 mm i.d.)	UV at 240 nm	ACN-water (35/65)	0.9	[19]
sildenafil	Chromolith® FlashRod (25 × 4.6 mm i.d.)	UV at 240 nm	Ammonium acetate (0.3 mol L ⁻¹)/ Acetonitrile (50:50)	2.4	[20]
Diclofenac	Chromolith® FlashRod (25 × 4.6 mm i.d.)	UV at 248 nm	MeOH-water (50/50)	2.4	[21]
Amoxicillin and clavulanic acid	Chromolith® FlashRod (25 × 4.6 mm i.d.)	UV at 228 nm	Phosphate (25 mmol L ⁻¹ , pH=3.0) / MeOH (85/15)	2.4	[22]

Table 1: Representative applications of sequential injection chromatography (SIC) to pharmaceutical analysis.

based on sol-gel techniques to achieve a homogeneous porous layer on a solid core of silica. It provided higher efficiencies due to the smaller diffusion distance and improved mass transfer, lower internal porosity and narrower particle size distribution (Figure 4) [27].

Figure 1: Typical structure of a reversed phase monolithic stationary phase.

Figure 2: Representative SIC setup: C = carrier (mobile phase); SP = syringe pump; HC = holding coil; SV = selection valve (or multiposition valve); MC = monolithic column; S = sample; W = waste; UV = detector.

Figure 3: SIC chromatogram of acyclovir and its major impurity guanine (from [23]).

Figure 4: Typical structure of core shell particles (from Phenomenex, Kinetex).

To conclude this editorial SIC is definitely a viable technique that offers enhanced potentials to chemical analysis. To my opinion SIC researchers should not focused on “competing” conventional HPLC to straightforward applications. The real strength of SIC is - as already

mentioned above - the possibilities of automated sample treatment (extraction, derivatization, dilution etc) and separation in a single run with a portable and low cost setup. In any case special attention should be paid to present fully validated analytical protocols that clearly prove their potentials to “real world” applications and especially in a demanding industrial environment.

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