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An overview of sequential injection chromatography

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ABSTRACT

New generation of sequential injection analysis (SIA) called sequential injection chromatography (SIC) has already been consolidated as a good alternative of high performance liquid chromatography (HPLC) for fast analysis of simple samples. Benefits of flow methods are automation, miniaturization and low sample and mobile phase consumption. Implementation of short monolithic chromatographic column into SIA opens new area—on-line chromatographic separation of multi-compound sample in low-pressure flow system, with the advantage of flow programming and possibility of sample manipulation. In the presented review the potential of SIC and its comparison with HPLC for determination of pharmaceutical mixtures is discussed and outlines past and recent trends focused on separation with SIC.

1. Introduction

In recent years, special attention was given for the development of automation for analytical procedures. A lot of new instrumental methods were described, sequential injection analysis (SIA) was one of them. Ruzicka and Marshall [1] proposed SIA as an advanced form of solution manipulation for mixing and transport of samples, reagents and products of chemical reactions (selective chemistry) to the measurement point for sample determination. This "single line" flow method brings many benefits for the routine analysis due to simple manifold, reduction of the reagents and sample consumption, short time of analysis and automation of analytical procedures [2]. The SIA is a high versatile technique based on a multi-position selection valve (enables precise filling of system with solutions) and double-direction pump (provides forward and reverse movement of sequentially built liquid stream in narrow tubes of the manifold) [3]. All mentioned features favor SIA technique for application in many fields of analysis, primarily for monitoring of long lasting processes and performing of more complicated operations [4–6].

2. Determination of mixtures by SIA

The problem of simultaneous determination of analytes in mixtures (presence of several compounds or complex matrix) can be solved by using of non-separation tools in SIA. The simplest example is the utilization of selective detectors (based on different characteristic of analytes in mixture) which was described for determination of complex formed between naproxen and β-cyclodextrin in pharmaceutical preparations using fluorescence detector without any previous separation, extraction or other sample manipulation [7] or for monitoring of glucose and penicillin during cultivations using specific reactions and chemiluminescence detection [8]. Organic acids and sugars in soft drinks were determined by Fourier transform infrared spectroscopy [9]. Ion selective electrodes sensitive to penicillin-G antibiotics were used for analysis

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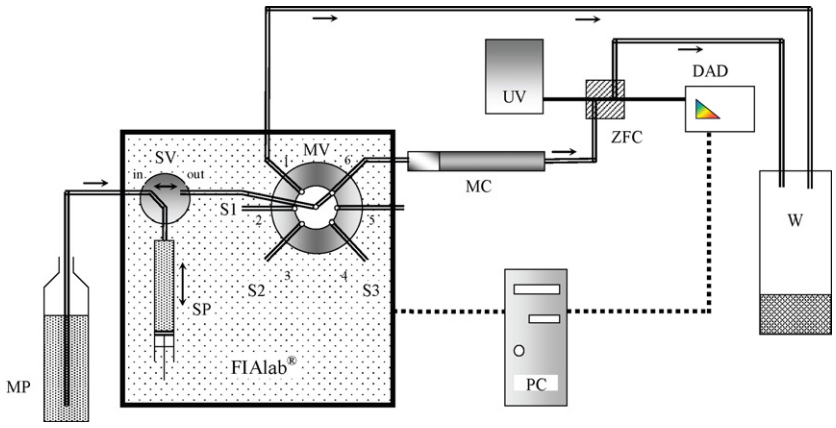

Fig. 1 – Scheme of SIC set-up for determination of SA and TCA. DAD: diode-array detector; MC: monolithic column; MP: mobile phase; MV: 6-port multi-position valve; SP: syringe pump; SV: solenoid valve; S1, S2, S3: sample 1, 2, 3; PC: computer, UV: UV lamp, W: waste, ZFC: Z-flow cell.

in pharmaceutical products [10]. Solid phase C18 restricted access material (RAM) columns was incorporated into SIA for direct determination of furosemide in human serum [11], selective anion exchange solid phase micro-column was described for simultaneous determination of benzophenone-4 and phenylbenzimidazole sulphonic acid in sunscreen sprays [12]. Lab-On-Valve (LOV) with a separation chamber was firstly introduced for liquid–liquid micro-extraction of diphenhydramine hydrochloride in pharmaceutical preparations and anionic surfactants in water samples [13]. Reduction cadmium column was described for simultaneous determination of nitrates and nitrites in water samples by Griess reaction [14–15]. Connection of gas-diffusion unit with SIA manifold was used for automatic determination of ammonium in environmental samples [16]. Dialyser with cellulose membrane enables sample dilution and matrix separation to measure pH and quantify chlorides by potentiometric detection with two ion-selective electrodes in a tubular configuration or nickel by colorimetric detection in electrolytic baths [17]. SIA manifold equipped with Lab-On-Valve was used for simultaneous spectrophotometric determination of copper and iron in wastewater [18] and for the determination of ultra-trace levels of Cd^{2+}, Pb^{2+} and Ni^{2+} by electrothermal atomic absorption spectrometry (with help of chelating Sepharose beads as sorbent material) [19]. Selective reaction of sulphate with barium–dimethylsulphonazo(III) for sulphate enables determination in automotive fuel ethanol [20]. Use of special mathematical models—multivariate curve resolution with alternating least squares was used for simultaneous determination of analytes in the presence of interferents without the need to pre-treat the sample [21].

3. Sequential injection chromatography—SIC

Coupling of short monolithic column with SIA opened a new possibility in flow analysis—it can easily solve problems with mixture analysis. The method firstly described in 2003 is called sequential injection chromatography (SIC) and has been already successfully applied in the analysis of relatively simple multi-component samples—mainly in the field of pharmaceutical analysis.

Sequential injection chromatography is built on classical SIA manifold (in our case FIAlab® 3000 or FIAlab® 3500 analyzers equipped with syringe pump). Chromatographic part is represented by short (usually 25 or 50 mm of length) commercially available monolithic column (with or without monolithic precolumn 5 or 10 mm length) placed between multi-position valve and Z flow cell of detector. Detection is provided by fiber optics UV–VIS DAD detector. Scheme of SIC manifold is depicted in Fig. 1.

4. Monolithic columns

The research in the field of liquid chromatography columns has tremendously accelerated during last years. Innumerable types of chromatography columns have been developed to solve particular problems of separation requirements. One important direction of this research area is the development of monolithic columns with high porosity sorbent (it permits high flow rates of mobile phase at low back pressures without losing efficiency) as a new separation tool used both in HPLC [22–23] and other flow analytical methods [24]. This feature can be utilized for integrating these columns into a SIA manifold (low pressure flow method with limited range of pump back-pressure to about 2.5 MPa) for extending the possibilities of this technique. Two brands of monolithic columns suitable for SIC are nowadays available commercially—Merck® Chromolith™ and Phenomenex® Onyx™ (in usable lengths of columns −25 mm or 50 mm are with silica based ODS–C18 sorbent only) [25–26]. The monolithic columns consist of a single piece of high-purity polymeric silica gel rod with a bimodal pore structure (macropores and mesopores—a porosity exceeding 80%). Macropores (average size 2 μm) dramatically reduce the column back-pressure and allow the use of higher flow rates. The mesopores (average size 13 nm)

form the fine porous structure and create the large uniform active surface enabling high performance chromatographic separation. Monolithic rod demonstrates very high mechanical stability and long operative lifetimes, in most cases far exceeding lifetime of particulate columns. Monolithic columns also exhibit similar chromatographic properties with respect to retention and selectivity as particle columns of the same specific surface area and pore diameter. Since monolithic columns became commercially available, they are being used in many different fields [27]. Another type of columns based on monolithic principle are Convective Interaction Media (CIM®) [28], a new polymeric macroporous material produced by radical co-polymerization and recently used for the separation of biomacromolecules.

5. Applications of SIC

Early works of SIC analysis were focused on the analysis of relatively simple multi-component samples as pharmaceuticals are. Solutions, drops, syrups, etc. without interfering incompatibilities and containing 2–5 compounds of interest can be determined directly only by diluting of sample by mobile phase. Other samples (topic creams, tablets, capsules, etc.) have to be pre-treated (for example by extraction into organic solvent). Length of column was chosen depending on chromatographic features of all substances in sample. Mobile phase was usually methanol or acetonitrile based and used amount was only volume able to elute all substances from column. Volume of syringe pump used in SIC system was 5.0 or 10.0 mL. Smaller syringe was more preferable due to the possibility to achieve higher working pressure in system. Flow rate of mobile phase was optimized according to the length of column and peak shapes (usually less than 1.5 mL min^{-1}). Detection by DAD UV–VIS detector (S 2000, Ocean Optics Inc., USA) was used coupled with Z flow cell with 10 mm active optical length. Two or three wavelengths were used simultaneously if it was necessary for increasing the detection sensitivity. Volume of sample used for one analysis was usually 10–20 μL depending on the column length. The whole system was controlled by commercial FIAlab® software with predefined sequence. The SIC served as good automatic analyzer for fast chromatographic determination of simple mixtures with possibility of easy handling and pre-treatment of sample. This feature decreases possibility of mistake of human factor and enables determination of aggressive or biohazard samples. SIC combined with Franz cell and peristaltic pump can be used with advantage to extend applicability for liberation tests of semi-solid pharmaceuticals through artificial skin or long time in vitro tests controlling changes of concentration of substances in time. Table 1 summarized relevant applications of SIC in pharmaceutical analysis.

5.1. *Liquid samples*

For determination of compounds in liquid mixtures is not usually necessary to do pre-treatment of sample. It is caused by fact that solutions are quite simple and adjuvants (ionic compounds) usually do not interfere with chromatographic measurement. Several new methods based on SIC were developed—for simultaneous determination of ambroxol, methylparaben and benzoic acid in various pharmaceutical syrups and drops with salicylic acid as internal standard [29], for determination of naphazoline nitras and conservant methylparaben and internal standard (ethylparaben) in eye drops (first time two wavelengths of UV detector simultaneously were used in order to increase the selectivity of the method) [30], SIC method for analysis of topical solution containing salicylic acid and triamcinolone acetonide with propylparaben as internal standard [31]. Only short and simple pre-treatment of sample (extraction by methanol with 1% of H_3PO_4) was performed before analysis of betamethasone and chloramphenicol in eye drops employing propylparaben as internal standard [32].

5.2. *Topical semisolid formulations*

Due to interfering adjuvants and matrix it is necessary to carry out simple and fast pre-treatment of topical semisolid formulations. Extraction with organic solvent has to be done before determination of topical cream containing triamcinolon acetonid, two conservants (methylparaben and propylparaben) and ketoprofen (internal standard) [33], before analysis of topical pharmaceutical preparations with salicylic acid, its ester methylsalicylate and propylparaben (internal standard) [34] and before determination of sodium diclofenac and conservants (methylparaben and propylparaben) in topical emulgel with butylparaben as internal standard [35].

5.3. *Tablets and capsules*

SIC was also used for chromatographic determination of ambroxol hydrochloride and doxycycline with ethylparaben as internal standard in pharmaceutical capsules [36] and for simultaneous determination of paracetamol, caffeine, acetylsalicylic acid and internal standard benzoic acid in common antipyretic and antiflogistic tablets [37]. Only simple and fast pre-treatment was used before analysis (extraction with organic solvent).

5.4. *Automation of simultaneous release tests*

Connection of SIC and Franz cell (functioning as standard vessel for controlling the liberation of active compounds from topical preparations) enabled to create fully automated system for the in vitro release testing of composed semisolid dosage forms. This system was used for determination of two active substances in topical pharmaceutical formulation composed of lidocaine and prilocaine and internal standard (trimecaine). Samples were taken in 10 min intervals during 4 h of the release test [38].

Efficiency of separation in SIC is in most cases comparable with HPLC thus ensure good results of determination. Typical SIC chromatogram of pharmaceutical mixture (Triamcinolon-IVAX topical solution) compared to HPLC chromatogram is depicted in Fig. 2. Chromatographic parameters of SIC process and HPLC process are in Table 2.

Table 1 – Applications of SIC

Matrix	Analytes	Column (mm)	Flow rate (mL min^{-1})	Mobile phase	Detection UV (nm)	Pre-treatment of sample	Time (min)	References
Pharmaceutical syrups and drops	Ambroxol, methylparaben, benzoic acid	50 + 10	0.48	Acetonitrile:tetrahydrofuran:water (10:10:90, v/v/v) pH 3.75 adjusted with triethylamine and acetic acid	245	Extraction by mobile phase	<11	[29]
Pharmaceutical drops	Naphazoline nitras, methylparaben	25 + 5	0.9	Methanol:water (40:65, v/v), pH 5.2 adjusted with triethylamine 0.8 μl $mL^{-1}$and acetic acid	220; 256	Dilution of drops	<4	[30]
Pharmaceutical drops	Triamcinolone acetonid, salicylic acid	50 + 5	0.9	Acetonitrile:water (35:65, v/v), pH 3.2 adjusted with acetic acid	240	Dilution of drops	<7	[31]
Pharmaceutical drops	Betamethasone, chloramphenicol	25 + 5	0.48	Acetonitrile:water (30:80, v/v)	241; 271	Exctraction by methanol with 1% of H_3PO_4	<8	[32]
Topical cream	Triamcinolone acetonid, methylparaben, propylparaben	25 + 10	0.6	Acetonitrile:methanol:water (35:5:65, v/v/v), pH 2.5 adjusted with 0.05% nonylamine and H_3PO_4	243	Extraction by methanol	<6	[33]
Topical cream	Salicylic acid, methylsalicylate	50	0.6	Acetonitrile:water (35:60, v/v), pH 2.5 adjusted with 98% H_3PO_4	240	Extraction by methanol	<7	[34]
Topical cream	Diclofenac natrium, methylparaben, propylparaben	25	0.48; 0.9; 1.2	Acetonitrile:water (40:70, v/v), pH 2.5 adjusted with 0.05% triethylamine and H_3PO_4	275	Extraction by methanol	<8	[35]
Pharmaceutical capsules	Ambroxol hydrochloride, doxycycline	25	0.48	Acetonitrile:water (20:90, v/v), pH 2.5 adjusted with 98% H_3PO_4	213	Exctraction by methanol with 1% of H_3PO_4	<9	[36]
Pharmaceutical tablets	Paracetamol, coffein, acetylosalicylic acid	25	0.6	Acetonitrile:water (10:90, v/v), pH 4.05 adjusted with 98% H_3PO_4	210	Extraction by methanol and mobile phase	<7	[37]
Topical cream	Lidocaine, prilocaine	25	0.6	Acetonitrile:water (40:80, v/v), pH 7.1 adjusted with 0.01% triethylamine and H_3PO_4	212	Automated system for the release testing of semisolid dosage forms	<7	[38]

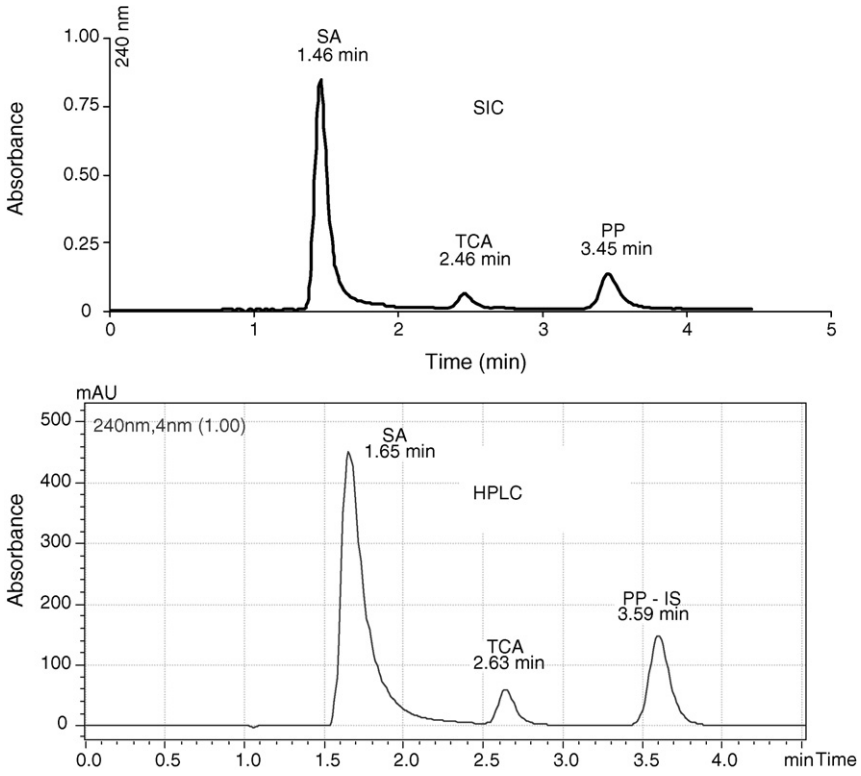

Fig. 2 – Comparison of SIC and HPLC chromatogram of the separation of active substances in Triamcinolon-IVAX topical solution. Mobile phase: acetonitrile/water (35:65, v/v), pH 3.3 adjusted with acetic acid, flow rate 0.9 mL min^{-1} at ambient temperature, UV detection at 240 nm [31].

Table 2 – Chromatographic parameters of SIC process and HPLC process of the separation of active substances (salicylic acid, triamcinolone acetonide and propylparaben-IS) in Triamcinolon-IVAX topical solution depicted in Fig. 2 [31]

	Salicylic acid		Triamcinolone acetonide		Propylparaben	
	SIC	HPLC	SIC	HPLC	SIC	HPLC
Peak symmetry	1.95	2.03	1.65	1.23	1.64	1.19
Number of theoretical plates	1475	812	2188	2620	3135	3085
	$R_{SA/TCA}$		$R_{TCA/PP}$			
Peak resolution	6.03	4.51	4.52	4.06		

6. Other chromatography approaches in SIA

Zacharis et al. [39] have incorporated monolithic strong anion-exchanger disk (CIM®) into SIA manifold for on-line drug–protein interaction studies. Two detection modes have been used—free fraction is monitored by its intrinsic fluorescent properties and retained BSA is under UV monitoring. Multisyringe flow system with three solenoid valves coupled with short monolithic column created by González-San Miguel et al. [40] have used for determination of β-lactam antibiotics. García-Jiménez et al. [41] have employed monolithic column in FIA for analysis of antioxidants in food, while Laver et al. [42] have used same system for determination of parabens in cosmetics.

7. SIC versus HPLC

SIC is promisingly evolving method that can be embedded both flow and chromatography methods and is augmenting potentialities of both scopes. SIC technique shows comparable separation efficiency and several advantages, compared to traditional HPLC. Short time of analysis and possibility of on-line use of reagents in all steps of determination, the production of the waste and the consumption of solvents are lower than in HPLC methods (due to discontinual flow), this enable reduction of cost per analysis (at least twice). Easy liquid manipulation not attainable by classical HPLC set up, dimensions of the SIC system and portability of analyzer provides the opportunity for analysis “on field”. Application of SIC is convenient for analysis

Table 3 – A comparison of different characteristics of HPLC and SIC

Characteristic	HPLC	SIC
Possibility of separation	Yes	Yes—simple mixtures[a]
Flow of mobile phase	Continual	Discontinual
Direction of flow of mobile phase	One way	Both ways + stop flow
Speed of mobile phase	Constant	Variable
Consumption of mobile phase	High	Low (discontinual flow)[b]
Use of reagents (reactions)	Limited[c]	Yes
Pre-treatment of sample	Yes (restricted)	Yes
Data evaluation	Sophisticated	Simple
Cost of instrumentation	High	Low[d]
Portability of analyzer	No	Yes[e]

[a] From two to five substances.
[b] Mobile phase is flowing during the separation only.
[c] Post-column derivatization.
[d] 2–3 time cheaper.
[e] Small-sized box.

of more simple mixtures containing preferably 2–5 analytes.

On the other side, SIC method is limited by column (only short monolithic) and maximal amount of mobile phase available for one analysis (available reservoir of syringe pump is at present only 5.0 or 10.0 mL). Robustness of SIC system used was not as high as a classic HPLC, mainly due to the limitation caused by the syringe pump used and back-pressure produced especially in higher flow rates. Commercial program for data processing and analyzing utilizes only height of peaks for quantification of response. A comparison of different characteristics of HPLC and SIC together with their advantages/disadvantages is shown in Table 3.

Recent development result in commercially available SIChrom™—accelerated liquid chromatography system (FIAlab® instruments, USA) that solves several weak points of the present SIC system. It is equipped with powerful pump (higher flow rates and use longer columns) and chemical resist Lab-On-Valve for variable sample handling and pre-adjustment [43].

8. Conclusion

In this review article application of chromatographic principles in SIA was discussed. The coupling of the commercial monolithic column (high separation performance at low pressure) with SIA manifold allows carrying out simple separations without an expensive instrumentation such as HPLC. SIC has been presented as convenient alternative HPLC for chromatographic determinations in pharmaceutical analysis with sufficient results. From this point of view, the monolithic silica columns opened a new area of separation analysis in highly flexible sequential injection systems. Moreover, the use of several (2–4) wavelengths simultaneously provides additional increasing of sensitivity of determination (measuring in wavelength of absorbance maximum of each compound). In summary, the SIC system can be an interesting tool for the rapid separation and quantification of several compounds not only in pharmaceutical analysis.

Further research will be focused on automation of pretreatment of biological samples (on-line extraction) and using other kinds of monolithic columns (different monolithic sorbents, lengths and diameters). Coupling of SIA with other selective detectors is another promising area (mass spectrometry detection, fluorescence detection, etc.), with the aim of increasing the sensitivity of determination. SIC has a great potential to be cheap and simple solution for everyday routine laboratory analysis. Portability of SIC analyzer provides the opportunity for analysis “on field”, which is not pursuable by classical HPLC set up.

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