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Short communication

Reversed-phase porous silica rods, an alternative approach to high-performance liquid chromatographic separation using the sequential injection chromatography technique

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Abstract

A commercially available porous silica rod column was used as a separation tool for the sequential injection analysis (SIA). A porous solid monolithic column showed high performance at a low pressure, allowing sequential injection analysis to be used for the first time for separation in HPLC fashion. In this contribution, we tried to demonstrate a new separation concept with SIA manifold for the simultaneous determination of four different compounds (methylparaben (MP), propylparaben (PP), triamcinolone acetonide (TCA) and internal standard ketoprofen (KP)) in a pharmaceutical triamcinolon cream 0.1% formulation. A Chromolith Flash RP-18e, 25 mm × 4.6 mm column with a 10 mm pre-column (Merck, Germany) and a FIAlab 3000 system (USA) with an 8-port selection valve and 10 ml syringe were used for sequential injection chromatographic separations in our study. The mobile phase used was acetonitrile–methanol–water (35:5:65, v/v/v) + 0.05% nonylamine, pH 2.5, flow rate 0.6 ml min^{-1}. The analysis time was <6 min. A novel sequential injection chromatography (SIC) technique with UV spectrophotometric detection was optimised and validated.

Keywords: Sequential injection analysis; Sequential injection chromatography; Monolithic columns; Injection methods; Instrumentation; Parabens; Pesticides; Triamcinolone acetonide

1. Introduction

In recent years, sequential injection analysis (SIA) has become an important analytical technique, mainly for the determination of drug in pharmaceuticals and for the determination of environmental contaminants.

SIA, proposed by Ruzicka and Marshall in 1990 [1], is based on forward, reversed, and stopped flow of the carrier stream and it has been the subject of several studies aimed to establish its theory and applications [2–5]. It offers several advantages: the instrumental set-up is very flexible, the mechanical components undergo little wear and hydrodynamic variables can be computer controlled with high efficiency. Mostly configured as a "single line system", the SIA device consists of a single low-pressure bi-directional syringe pump as flow drive that gener-

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ates main pulsation-less flow with variable direction and speed, and a multi-position selection valve both as injector of samples and reagents and as connector with reactors and detector. During measurement, selected volumes of sample and reagent solutions are drawn into the holding coil by flow reversals and then moved forward through the selection valve into the detector. All the operations connected with liquid handling can be automated using automated syringe pump as a liquid driver and selection valve.

On the other side, the SIA technique has an important drawback—lacks the ability to carry out the separation analysis of multicomponent samples.

In this contribution, we developed a new low-pressure separation concept of chromatography—sequential injection chromatography (SIC) with SIA manifold. Programmable flow, provided by SIA system results in low organic solvent consumption, low operating costs, low generation of the waste and relatively high sample throughput. The 8-port selection valve allows to combine a number of mobile phases (reagents); the syringe pump makes possible to change the flow rate and direction according the computer program.

Monolithic supports have become the subject of extensive study in the past years and they were developed based on a new sol–gel process, which includes the hydrolysis and polycondensation of alkoxysilanes [6–10].

In contrast to conventional HPLC columns, the monolith columns are formed from a single piece of porous silica gel. These columns exhibit 90% interstitial porosity as compared to 80% in case of packed columns due to the presence of large through pores [7]. The resulting column back-pressure is therefore much lower and it allows incorporation into the SIA system. The back-pressure limit range of the syringe pump is about 2.5 MPa.

Triamcinolone acetonide (TCA) is an anti-flogistic and anti-allergic drug used frequently in therapy of rheumatic, allergic and dermatologic symptoms [11].

Methylparaben (MP) and propylparaben (PP) are effective antibacterial and antifungal agents, which are commonly used as preservatives in foods, beverages, cosmetics and pharmaceuticals. Methylparaben and propylparaben are used together since they have a synergic effect [12].

In the literature there has been recently a number of reports dealing with various analytical methods for the determination of TCA, mainly by HPLC methods [13–15], but there is no method for simultaneous determination of TCA in the presence of MP and PP.

2. Experimental

2.1. Materials and apparatus

The standard of TCA was obtained from Sicor (Milan, Italy), MP and PP were from Galena (Opava, Czech Republic), and internal standard ketoprofen (KP) was from Sigma–Aldrich (Prague, Czech Republic). Standard solutions were prepared in methanol. The final concentrations of the sample or reference standards were 50 $\mu g\,ml^{-1}$ of TCA, 100 $\mu g\,ml^{-1}$ of MP, 25 $\mu g\,ml^{-1}$ of PP and 50 $\mu g\,ml^{-1}$ of internal standard KP.

Methanol and acetonitrile (Chromasolv, for LC) were obtained from Sigma–Aldrich, orthophosphoric acid, 85% (analytical-reagent grade) was from Merck (Darmstadt, Germany). Nonylamine was obtained from Fluka. Triamcinolon cream 0.1% was supplied from Herbacos-Bofarma (Bochemie Group, Pardubice, Czech Republic).

An overall schematic view of the sequential injection chromatography system with the monolithic column is shown in Fig. 1. A FIAlab 3000 system (FIAlab Instruments, USA) is a commercially produced instrument and consists of a syringe pump (syringe reservoir 10 ml) and 8-port selection Cheminert valve (Valco, USA). FIAlab 3000 was equipped with fiber-optic UV-VIS diode array detector S2000 (Ocean Optics Inc., USA) with UV-Vis tungsten lamp LS-1 (Ocean Optics Inc., USA). The solarisation optic fibers and 10 mm Z-flow cell were from Avantes (Colorado, USA). The whole SIA system was controlled by the latest version of program FIAlab for Windows 5.0. Flow lines were made of 0.75 mm i.d. PTFE tubing. Mobile phases and samples were aspirated through the selection valve and then delivered to the monolithic column and to the detector. Sample compounds separation was performed on Chromolith Flash RP-18e, 25 mm × 4.6 mm column (Merck) with 10 mm pre-column. The monolithic column was placed between the selection valve and flow cell of the detector.

The comparative HPLC system, consisting of a binary pump LCP 4100 (Ecom, Prague), Waters

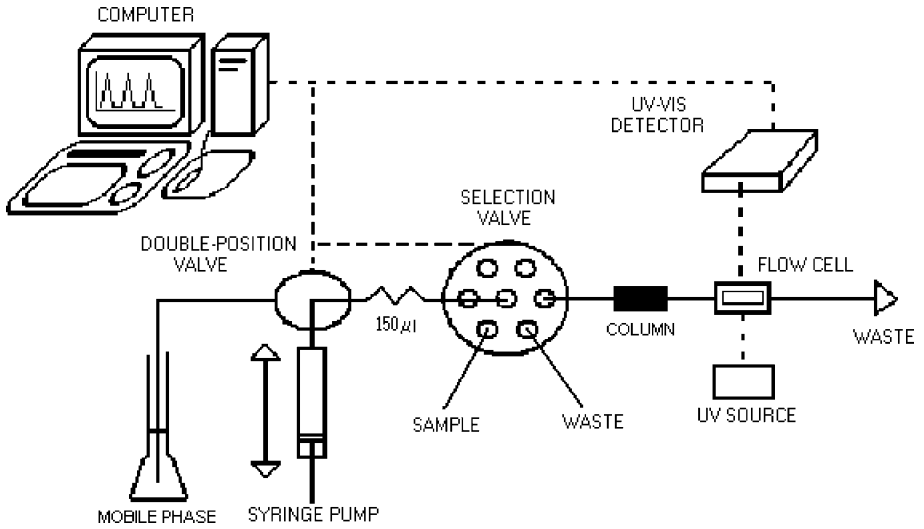

Fig. 1. Scheme of SIC set-up for chromatographic separation and determination of triamcinolone acetonide, methylparaben and propylparaben.

autosampler 717 plus, UV detector Waters 486 (Waters, Milford, MA, USA) and chromatographic software CSW v.1.7 for Windows (Data Apex s.r.o., Prague, Czech Republic). Analyses were performed on the same above mentioned column.

2.2. Methods

An accurately weighed portion (ca. 0.5 g) of the pharmaceutical emulgel was transferred into a 50 ml centrifuge tube and supplemented with 20.00 ml of internal standard (50 $\mu g\,ml^{-1}$ of KP in methanol). The mixture was placed into the ultrasonic bath for 10 min and then centrifuged at 4000 $\times$ g for 10 min. A volume of 10 μl of supernatant was analysed by SIA-chromatography system. Identification of peaks in the emulgel samples was based on comparison of retention times of compounds in standard solutions.

The optimal mobile phase for separation of TCA, MP, PP and internal standard KP was acetonitrile–methanol–water (35:5:65, v/v/v) + 0.05% nonylamine, pH adjusted to 2.5 by means of orthophosphoric acid (85%). Mobile phase was degassed before application by means of helium.

The finally selected optimised conditions were as follows: injection volume 10 μl for sample of emulgel, the isocratic mobile phase was pumped at flow rate 0.6 $ml\,min^{-1}$ at ambient temperature, and the detection wavelength was 243 nm. Fig. 2 shows a sequential injection chromatogram of compounds of pharmaceutical cream with KP as internal standard.

3. Results and discussion

3.1. Method development and optimisation

The main aim of our study was to find optimal separation condition of the sequential injection cycle for simple method of determination of TCA, MP and PP, and to explore suitability of this method application for real pharmaceutical sample. The method should be able to separate all compounds of interest and should be free of interference from excipients, and be robust and straightforward enough for routine use in determination of compounds in topical emulgel.

The optimal detection wavelength was chosen to be 243 nm, the absorption maximum of TCA.

The choice of appropriate internal standard was made from several compounds (butylparaben, flurbiprofen, sodium diclofenac and ketoprofen). Ketoprofen was sufficiently separated from other compounds of interest and did not prolong the time of the analysis.

For successful separation of TCA, MP and PP, the different mobile phases were tested (methanol, ace-

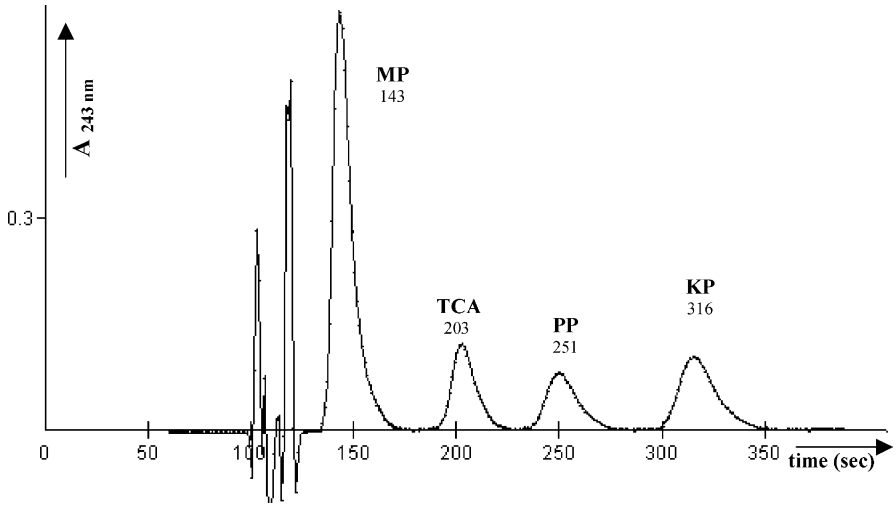

Fig. 2. Sequential injection chromatogram of all compounds in a topical cream triamcinolone acetonide 0.1%. The content of MP 0.2%, content of TCA 0.1% and content of PP 0.05%.

tonitrile and water). Using different column lengths Chromolith Flash RP-18e, 25 mm × 4.6 mm and Chromolith SpeedROD RP-18e, 50 mm × 4.6 mm were tested. The similar retention characteristics of compounds allowed selection of the 25 mm column with 10 mm pre-column for the separation of target compounds with sufficient resolution. The optimal mobile phase for 35 mm total column length was acetonitrile–methanol–water (35:5:65, v/v/v) + 0.05% nonylamine, pH 2.5, flow rate 0.6 ml min^{-1}. In spite of the triamcinolone acetonide is not basic compound, the addition of nonylamine to the mobile phase resulted in decreasing of peak tailing and better peak symmetry. The total volume for one analysis was 3.6 ml of mobile phase with the time less than 6 min.

3.2. Analytical parameters and validation

The optimised method was validated by a standard procedure. Accuracy was determined using spiked placebo solutions, two preparations each, two injections of each preparation. Relative standard deviation (R.S.D.) values were calculated for repeated standard injections (system precision) as well as repeated injections of multiple sample preparations (method precision). Visual inspection of chromatograms of standards and placebo solutions was conducted to ensure the selectivity of the method. None interference peak was found in the retention time of all compounds after placebo sample injection. The method validation results obtained under the final conditions are shown in Table 1.

The optimised SIC method was evaluated from the parameters of chromatographic process point of view: the relative standard deviation of retention times for intra day repeated standard injections ($n = 7$), the peak resolution, peak asymmetry and separation efficiency for all compounds were calculated. The obtained results are shown in Table 2. The chromatogram in Fig. 2 was obtained using the SIC method with sample of cream. The comparison with HPLC, in Fig. 3, was carried out under the same conditions as well as the SIC system. Small difference in retention times between SIC system and HPLC is caused by different dead volumes of the systems and by different inner diameters of flow lines.

3.3. Determination in a pharmaceutical product

All compounds present in the sample of topical triamcinolon cream 0.1%, TCA, both preservatives—MP, PP, and internal standard were clearly separated and quantified. The optimal extraction medium was found to be the methanol. The average amounts of TCA, MP and PP in pharmaceutical cream were 96.5 ± 4.0%, 97.4 ± 3.2% and 98.3 ± 2.0% of the

Table 1
The analytical parameters and the method validation results

	Methylparaben	Triamcinolone acetonide	Propylparaben
Calibration range ($\mu g\,ml^{-1}$)[a]	1–40	2–100	2–60
Correlation coefficient	0.99962	0.99968	0.99952
Limit of detection ($\mu g\,ml^{-1}$)[b]	0.25	0.5	0.25
Limit of quantification ($\mu g\,ml^{-1}$)	0.75	1.5	0.75
System precision (%)[c]	2.00; 2.08; 2.05	1.37; 1.04; 2.16	1.27; 0.85; 2.21
Method precision (%)[d]	2.32	1.92	0.39
Accuracy			
Spike recovery (%)[e]	97.1	97.39	101.24
Recovery R.S.D. (%)	1.56	3.54	0.94

[a] Each concentration was measured in triplicate ($n = 9$).
[b] Volume of sample injection—50 μl of standard solution in mobile phase.
[c] Relative standard deviation (R.S.D.) values were calculated for repeated standard injections ($n = 8$) at three concentration levels $c = 2$, 5, and 40 $\mu g\,ml^{-1}$, respectively.
[d] R.S.D. for repeated injections of multiple sample preparations ($n = 6$), two preparation each, two injection of each preparation.
[e] Spiked placebo solutions ($n = 6$)—two preparations each, two injections of each preparation.

Table 2
The parameters of SIC process

	MP	TCA	PP	Internal standard KP
Retention time (s)	143	203	251	316
Repeatability of t_R (%)[a]	0.06	1.13	1.25	0.62
Peak resolution	$R_{MP,TCA} = 5.7$	$R_{TCA,PP} = 3.9$	$R_{PP,KP} = 4.6$	
Number of theoretical plates	1110	2190	1975	2420
Peak asymmetry	1.73	1.35	1.40	1.44

[a] Repeatability of t_R—R.S.D. of retention times for intra day repeated standard injections ($n = 7$).

labelled amount, respectively. These results are in a good agreement with the pharmacopoeia requirements on the active compound content in the pharmaceutical topical preparations (range 95.0–105.0%).

Results of assays in the SIC system were compared with the conventional HPLC determination and there was found no significant difference (the statistical *t*-test (95% level)) between the average values of both

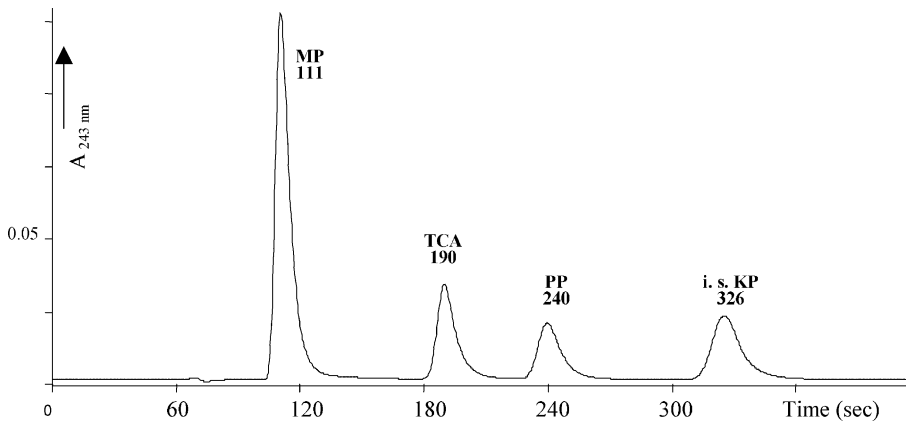

Fig. 3. HPLC chromatogram of the separation of standard solutions under the same conditions as well as the SIC system.

methods. The SIC method was found to be applicable for the routine analysis of the active compound triamcinolone acetonide and preservatives in a pharmaceutical triamcinolon cream 0.1%.

4. Conclusion

This work introduces a novel approach to multicomponent separations and highlights advantages and disadvantages of the coupling of monoliths with SIA.

In summary, the simple and automated system that was developed presents several clear advantages over the present methodologies: possibility of separation analysis without HPLC instrumentation, possibility of simple pre- or post-column derivatisation (the commercially SIA system is equipped with the programmable peristaltic pump), low consumption of the organic solvents and resulting low waste production due to the non-continuous flow. Moreover, the cost of the commercially available system (FIAlab Instruments, USA), is about one third equipment purchase price of the common HPLC instrumentation.

The disadvantages of the system, at present, are limitation of software for separation analysis evaluation (compared with the common HPLC software), less robustness than HPLC, limited flow rates of the syringe pump due to maximum back-pressure about 2.5 MPa, limited volume of the syringe pump (commercially available maximum is 10 ml), limited range of the separation due to the restriction of the column length and flow rate.

In the comparison between the other separation methods, SIC shows the above mentioned restrictions. On the other hand, conventional separation techniques are reliable and sensitive and require more expensive instrumentation and higher cost per assay.

In summary, our experience with SIC system shows the future use of SIC is mainly for the simple separation analysis of samples containing maximum of two to five compounds. However, coupling of the monolithic columns with the sequential injection system provides another tool to solve the simple separation problems rapidly and efficiently without need for HPLC instrumentation.

Acknowledgements

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