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Short communication

A novel application of Onyx™ monolithic column for simultaneous determination of salicylic acid and triamcinolone acetonide by sequential injection chromatography

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Abstract

A novel and fast simultaneous determination of triamcinolone acetonide (TCA) and salicylic acid (SA) in topical pharmaceutical formulations by sequential injection chromatography (SIC) as an alternative to classical high performance liquid chromatography (HPLC) has been developed. A recently introduced Onyx™ monolithic C18 (50 mm × 4.6 mm, Phenomenex®) with 5 mm monolithic precolumn were used for the first time for creating sequential injection chromatography system based on a FIAlab® 3000 with a six-port selection valve and 5.0 mL syringe pump in study. The mobile phase used was acetonitrile/water (35:65, v/v), pH 3.3 adjusted with acetic acid at flow rate 0.9 mL min^{-1}. UV detection provided by fibre-optic DAD detector was set up at 240 nm. Propylparaben was chosen as suitable internal standard (IS). There is only simple pre-adjustment of the sample of topical solution (dilution with mobile phase) so the analysis is not uselessly elongated. Parameters of the method showed good linearity in wide range, correlation coefficient >0.999; system precision (relative standard deviation, R.S.D.) in the range 0.45–1.95% at three different concentration levels, detection limits (3σ) 1.00 μg mL^{-1} (salicylic acid), 0.66 μg mL^{-1} (triamcinolone acetonide) and 0.33 μg mL^{-1} (propylparaben) and recovery from the pharmaceutical preparations in the range 97.50–98.94%. The chromatographic resolution between peaks of compounds was more than 4.5 and analysis time was 5.1 min under the optimal conditions. The advantages of sequential injection chromatography against classical HPLC are discussed and showing that SIC can be a method of option in many cases.

Keywords: Sequential injection chromatography, SIC; ONYX™ monolithic column; Salicylic acid; Triamcinolone acetonide; Spectrophotometric determination

1. Introduction

Chromatographic columns are widely used in separation of mixtures in almost all branches of science. Sequential injection chromatography (SIC) based on integration of short monolithic column into flow manifold is a new generation of flow methods analysis and extending the possibilities of sequential injection analysis (SIA) technique enabling determination of simple multi-compound samples in one step without pretreatment of sample.

SIA—high versatile technique based on a programmable flow [1] has been proposed by Ruzicka and Marshall as an efficient tool for automated liquid handling [2]. The "single-line" technique is based on forward and reverse movement of a piston of syringe pump, which together with a multi-position selection valve enables precise sampling of liquid chemicals into the system and propelling of the sequenced zones to the reactors and detector [3]. Automation, speed of the analysis and low consumption of sample and reagents are the most important features that favour the SIA technique for application in many fields of analysis, primarily by more complicated operations such as sample pre-treatment, derivatisation reactions or monitoring of long lasting processes [4–6].

On the other side, SIA technique itself has generally one important drawback—it cannot primary provide the separation procedure and analysis of multi-component samples. This weak point was solved by coupling of short monolithic column [7] with SIA manifold and created method called sequential injection chromatography. It was recently successfully applied in the analysis of relatively simple multi-component samples [8–13].

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The use of monolithic columns (formed by "Sol–gel" technology from a single piece of porous silica gel) enabling operation at high flow rates with lower back-pressure [14]). This feature can be used for integrating these columns into a SIA manifold and for extending the possibilities of SIA technique (the back-pressure limit range of the syringe pump in SIA apparatus is about 2.5 MPa).

Salicylic acid (SA) is the traditional pharmaceutical substance. It has antiseptic and antifungal properties. It shows keratoplastic and keratolytic effect, which is often used in the topical preparations in combination with other substances, e.g. prednisolone, estradiol, betamethasone, triamcinolone, etc. It is also a product of decomposition of acetylsalicylic acid, which is a common antiinflammatory, analgesic and antipyretic drug used in topical preparations and tablets. Triamcinolone acetonide (TCA) is an antiflogistic and antiallergic drug used frequently in therapy of rheumatic, allergic and dermatologic symptoms.

There have been recently a number of reports in literature dealing with various analytical methods for the determination of SA [15–16] and TCA [17–19], mainly by HPLC methods, but there is no analytical method in literature for simultaneous determination of TCA in the presence of SA.

2. Experimental

2.1. Apparatus

2.1.1. Sequential Injection system and monolithic column

A commercially available instrument FIAlab® 3000 system (FIAlab® Instruments Inc., Bellevue, USA) with a syringe pump (syringe reservoir 5.0 mL) and a six-port selection Cheminert® valve (Valco Instrument Co., Houston, USA) was used. The manifold was equipped with fibre-optic UV–VIS diode array detector USB 2000 (Ocean Optics Inc., Dunedin, USA), 10 mm Z-flow cell (Avantes, Colorado, USA) and UV light source D-1000-CE (Analytical Instrument Systems Inc., Flemington, USA). The whole SIC system was controlled by the 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 six-port multi-position valve then delivered to the monolithic column and to the detector. Direct sample separation was performed on C-18 monolithic column Onyx™ Monolithic C18 (50 mm × 4.6 mm) (Phenomenex®, USA) coupled with monolithic precolumn (5 mm × 4.6 mm) (Phenomenex®). The monolithic column with precolumn was placed between the multi-position valve and flow cell of the detector [9].

2.1.2. HPLC apparatus

Preliminary experiments concerning the composition of mobile phase for precise separation and comparative measurement of pharmaceutical product samples were performed on HPLC system Shimadzu Prominence 20 series with DAD detector. Analyses were performed on the same above-mentioned column. Mobile phases of different composition were tested at flow rate 0.9 mL min^{-1} and the detector was set on 240 nm.

2.2. Reagents

Salicylic acid was obtained from Herbacos-Bofarma Ltd. (Czech Republic, Ph.Eur.3 grade quality), triamcinolone acetonide was obtained from Sicor (Milan, Italy, Ph.Eur.3 grade quality), propylparaben was obtained from Zentiva, a.s. (Czech Republic, Ph.Eur.3 grade quality) and organic modifiers for mobile phase preparation were obtained from Sigma–Aldrich. Acetonitrile (Chromasolv, for LC) was obtained from Sigma–Aldrich. All other used chemicals were of analytical grade quality. Millipore Milli-Q RG (Millipore s.r.o., Czech Republic) ultra pure water was used for preparing the solutions. Mobile phases were degassed by helium before use. Stock standards were solved in acetonitrile 60% (v/v) at concentration of 20 mg mL^{-1} (SA), 2 mg mL^{-1} (TCA) and 4 mg mL^{-1} (PP)—all were stored at 5 °C for 1 month. The final concentrations of the sample working standard solutions or reference standards for pharmaceutical preparations analysis were prepared by diluting the stock solution in the mobile phase.

2.3. Method and sample preparation

2.3.1. Mobile phase

The optimal mobile phase for separation of SA, TCA and internal standard (IS) propylparaben (PP) was acetonitrile–water (35:65, v/v), pH 3.3 adjusted with acetic acid; flow rate 0.9 mL min^{-1} at ambient temperature. Volume of mobile phase used for one analysis was 4.0 mL.

2.3.2. Solutions and sample preparation

The tested pharmaceutical preparation was Triamcinolon-IVAX (IVAX, Czech Republic) containing 20 mg of SA and 2 mg of TCA in 1.0 mL of topical solution. Sample adjustment was done by the following procedure: 100.0 μL of Triamcinolon-IVAX solution was transferred to 10.00 mL calibrated flask; then 100.0 μL stock solution of internal standard propylparaben was added and the flask was filled to the mark with mobile phase and mixed. The sample was homogenized and dissolved by 5 min sonication. The comparative standard solution was same for all the analysis and it was prepared diluting stock standard solution in 10.00 mL calibrated flask and the flask was filled to the mark with mobile phase and mixed. The final concentrations of the analytes in comparative standard solution were 200.0 μg mL^{-1} of SA, 20.0 μg mL^{-1} of TCA and 40.0 μg mL^{-1} of PP. Standards and samples were measured in triplicates and the mean peak height values were used for data acquisition.

3. Results and discussion

3.1. Method development and optimization

Applications of SIC has already been introduced in our previous papers [7–13]. In this work for the first time has been used Onyx™ (Phenomenex®) column instead of Chromolith™ (Merck®) column in the SIC method. The optimization of mobile phase was started with Onyx™ Monolithic C18

Table 1
Characterization of SIC process

	Salicylic acid		Triamcinolone acetonide		Propylparaben	
	SIC	HPLC	SIC	HPLC	SIC	HPLC
Retention time (min)	1.46	1.65	2.64	2.63	3.45	3.59
Peak symmetry	1.95	2.03	1.65	1.23	1.64	1.19
Number of theoretical plates	1475	812	2188	2620	3135	3085
Peak resolution	$R_{SA/TCA} = 6.03$	$R_{SA/TCA} = 4.51$	$R_{TCA/PP} = 4.52$	$R_{TCA/PP} = 4.06$		

(50 mm × 4.6 mm, Phenomenex®) with 5 mm monolithic precolumn and acetonitrile/water (50:50, v/v) mobile phase. The mobile phases containing acetonitrile as organic part have shown better separation of peaks and peak symmetry than methanol containing mobile phases. The optimization was focused to make a proposal appropriate ratio of acetonitrile/water composition and optimal pH of mobile phase to achieve a sufficient symmetry of the peaks together with a good separation of compounds and a short retention time. The optimal mobile phase for the separation of SA, TCA and internal standard was found acetonitrile/water (35:65, v/v), pH adjusted to 3.3 by means acetic acid. Flow rate was set to 0.9 mL min^{-1}. Propylparaben fulfills the requirements so was chosen as a suitable internal standard. The sample injection volume was 10.0 μL. Due to very different quantity of SA and TCA in pharmaceutical formulation (sample of pharmaceutical solution contains ten time more SA than TCA) only one wavelength (240 nm, absorption maximum of TCA in mobile phase) was used to ensure high absorption response of TCA and lower response of SA (not exceed range of detector). The proposed system enabled successful separation of target analytes in the time 5.1 min (0.66 min for aspiration of mobile phase, 0.0016 min for aspiration of sample and 4.43 min for elution of sample) and total volume of mobile phase for one analysis was 4.0 mL. Peak height evaluation was performed using the FIAlab® software.

3.2. Parameters of sequential injection chromatography process

The target compounds were successfully separated using the proposed procedure. Basic chromatographic parameters were calculated from experimental data, such as retention times, peak symmetry, number of theoretical plates and peak resolution; they are given in Table 1 in comparison to HPLC results.

3.3. Validation and analytical parameters of the method

The validation and analytical parameters (linearity, sensitivity, repeatability, recovery, selectivity, precision and accuracy) have shown good results. Linearity was established with a series of working solutions prepared by diluting the stock solution with mobile phase to the final concentrations. Each concentration was injected in triplicate and the mean value of peak height was used for the calibration curve. The calibration graphs involved seven experimental points for SA (concentration range 3.1, 6.3, 12.5, 25.0, 50.0, 100.0 and 300.0 μg mL^{-1}) and it is described by the following equation: $A = (0.0035 \pm 0.00001)c + (0.0018 \pm 0.0011)$ (where A is the absorbance and c the analyte concentration), the correlation coefficient was 0.9999; for TCA and PP nine experimental points (concentration range 6.2, 12.3, 18.5, 27.7, 41.6, 83.0, 125.0, 166.0 and 250.0 μg mL^{-1}) and they are described by following equations: TCA $A = (0.0034 \pm 0.00003)c - (0.0096 \pm 0.0031)$, the correlation coefficient was 0.9998; and for internal standard PP: $A = (0.0029 \pm 0.00002)c - (0.0062 \pm 0.0027)$, the correlation coefficient was 0.9998. The limit of detection (LOD) was calculated by comparison of the three-fold variation of signal to noise ratio (3 S/N) obtained from analysis of the standards, and the limit of quantification (LOQ) was defined as the lowest measured quantity above which the analyte can be quantified at a given statistical level of (3 LOQ). The system precision

Table 2
Analytical parameters and method validation results

	Salicylic acid	Triamcinolone acetonide	Propylparaben
Calibration range (μg mL^{-1})	3.1–300.0	6.2–250.0	6.2–250.0
Correlation coefficient	0.9999	0.9998	0.9998
Limit of detection 3σ (μg mL^{-1})	1.00	0.66	0.33
Limit of quantification (μg mL^{-1})	3.00	2.00	1.00
System precision–concentration 200 μg mL^{-1} (%)[a]	0.64	0.47	0.44
System precision–concentration 100 μg mL^{-1} (%)[a]	0.91	0.96	0.50
System precision–concentration 10 μg mL^{-1} (%)[a]	1.28	1.49	1.95
Repeatability of T_R (%)[a]	0.46	0.42	0.47
Method precision (%)[b]	1.73	3.43	
Accuracy–spike recovery (%)[c]	98.9	97.5	

[a] Relative standard deviation (R.S.D.) values were calculated for repeated standard injections ($n = 8$).
[b] R.S.D. for repeated injections of multiple sample preparations ($n = 6$).
[c] Spiked sample solutions.

of the method was determined by preparing the standards of SA, TCA and PP at three concentration levels (200.0, 100.0 and 10.0 μg mL^{-1} of all standards) and peak heights for each compound were determined after processing each standard eight times. The method validation results obtained under the final conditions are shown in Table 2. The method was found to fulfill common requirements of accuracy, precision and linearity (calibration range with correlation coefficient >0.999, R.S.D. for repeated standard injections at three concentration levels ($n = 8$) was less than 1.6%). To validate the precision of the method a number of six different pharmaceutical sample solutions were used, which were prepared from the same batch and analyzed consecutively. This approach provides a means of covering the precision of the entire method, from sample preparation to data handling. The precision of the method calculated as R.S.D. of six sample determination, including sample preparation, was 1.73% for SA and 3.43% for TCA. The mean values of found amount were 99.41% of SA and 97.29% of TCA. The accuracy of the method was carried out measuring of the pharmaceutical samples fortified with known quantity of the analytes (addition of 20% amount of SA standard and 100% amount of the TCA and PP standards to pharmaceutical preparation). Spiked sample solutions and un-spiked sample solutions were compared for recovery evaluation. The method accuracy results—mean values of the recoveries were found as 98.9% for SA and 97.5% for TCA. Assay values of recoveries show that the method allows direct determination of SA and TCA in commercial dosage forms in the presence of other adjuvants (analysis of blank solution of matrix showed no interferences in measurement, depicted in Fig. 1).

3.4. Determination in pharmaceutical product

The novel method developed has been applied to the determination of SA and TCA in topical solution Triamcinolon-IVAX. The pharmaceutical was commercially available on the Czech market. The interference effect of adjuvants (EDTA and carbethopendecinii bromidum) was not observed. The samples were prepared just by dilution hundred times with mobile phase containing IS. For analysis, a loop of three loads of comparative standard solution was programmed and three loads

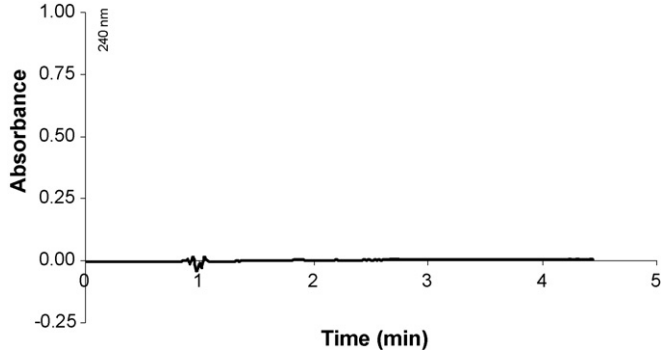

Fig. 1. SI chromatogram of blank solution of matrix of Triamcinolon-IVAX. Mobile phase: acetonitrile/water (35:65, v/v), pH 3.3 adjusted with acetic acid, flow rate 0.9 mL min^{-1} at ambient temperature, UV detection at 240 nm.

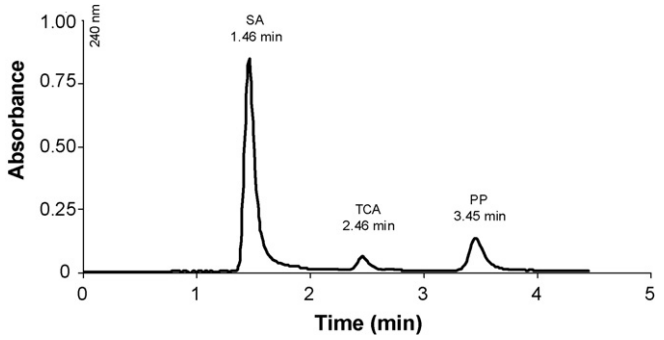

Fig. 2. SI chromatogram of the separation of active substances of Triamcinolon-IVAX topical solution. Mobile phase: acetonitrile/water (35:65, v/v), pH 3.3 adjusted with acetic acid, flow rate 0.9 mL min^{-1} at ambient temperature, UV detection at 240 nm.

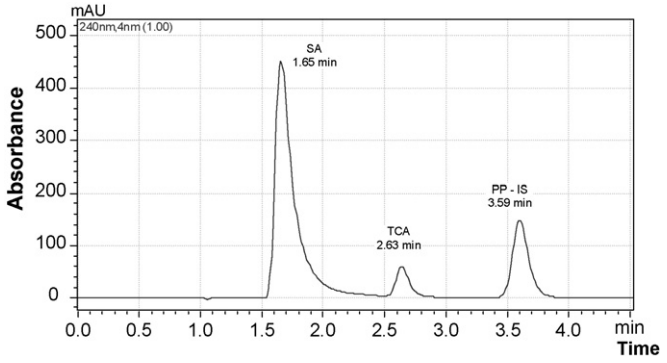

Fig. 3. HPLC chromatogram of the separation of active substances of Triamcinolon-IVAX topical solution. Mobile phase: acetonitrile/water (35:65, v/v), pH 3.3 adjusted with acetic acid, flow rate 0.9 mL min^{-1} at ambient temperature, UV detection at 240 nm.

of sample solution. The results of determination of six samples (different batch) were in range (99.32–101.43%; R.S.D. 1.83) for SA and (100.32–107.50%; R.S.D. 2.55) for TCA of declared value. The procedure of sample preparation was simple, fast and achieving high precision and low sample and reagent consumption. Representative sequential injection chromatogram showing successful separation of active substances of Triamcinolon-IVAX including internal standard is shown in Fig. 2.

After the SIC determination was done comparative HPLC measurement of mentioned six samples of Triamcinolon-IVAX solution with comparable results (SA 99.61–102.77%; R.S.D. 1.20% and TCA 100.64–104.18%; R.S.D. 1.32%) at the same conditions. Representative HPLC chromatogram showing successful separation of active substances of Triamcinolon-IVAX including internal standard is shown in Fig. 3.

4. Conclusion

Recently it has been shown that the novel SIC system as the solution-handling and automation system enables fast determination of simple mixtures and gives additional possibilities in chromatographic measurements compared with the conventional HPLC. An Onyx^{TM} Monolithic C18 (50 mm × 4.6 mm,

Phenomenex®) column with 5 mm monolithic precolumn incorporated first time into SIC manifold was proved to be a convenient and efficient tool for the separation and determination of salicylic acid and triamcinolone acetonide in pharmaceutical preparation (topical solution). Even in case of diametrical difference of amount of both compounds (proved solution contains ten times more SA than TCA) the analysis was achieved simultaneously only with simple pre-treatment of sample (dilution with mobile phase). Results of many chromatographic parameters for determination SA and TCA in pharmaceutical solution were compared to HPLC measurements. SIC technique shows several advantages as short time of analysis, easy handling with sample and low consumption of sample and solvents and low production of the waste (due to discontinual flow). These features enable reduction of cost per analysis. 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".

On the other hand, SIC is limited by column (at present only short monolithic reverse phased) and limited amount of mobile phase available for one analysis due to the use of 5.0 mL syringe pump. Commercial program for data processing can only height of peaks evaluate. Recent development results in commercially available SIChrom™ liquid chromatography analyzer by FIAlab® Instruments [20] that can solve several weak points of the present SIC system—equipped with more powerful pump (enables use of higher flow rates and longer columns) and chemical resistant Lab-On-Valve system for variable sample handling.

In summary, the SIC system renders a useful alternative to existing chromatographic methods and can be an important tool for the rapid separation and quantification of several compounds not only in pharmaceutical analysis.

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