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Determination of ambroxol hydrochloride, methylparaben and benzoic acid in pharmaceutical preparations based on sequential injection technique coupled with monolithic column

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Abstract

The porous monolithic columns show high performance at relatively low pressure. The coupling of short monoliths with sequential injection technique (SIA) results in a new approach to implementation of separation step to non-separation low-pressure method.

In this contribution, a new separation method for simultaneous determination of ambroxol, methylparaben and benzoic acid was developed based on a novel reversed-phase sequential injection chromatography (SIC) technique with UV detection.

A Chromolith® SpeedROD RP-18e, 50-4.6 mm column with 10 mm precolumn and a FIAlab® 3000 system with a six-port selection valve and 5 ml syringe were used for sequential injection chromatographic separations in our study. The mobile phase used was acetonitrile–tetrahydrofuran–0.05 M acetic acid (10:10:90, v/v/v), pH 3.75 adjusted with triethylamine, flow rate 0.48 ml min^{-1}, UV-detection was at 245 nm. The analysis time was <11 min.

A new SIC method was validated and compared with HPLC. The method was found to be useful for the routine analysis of the active compounds ambroxol and preservatives (methylparaben or benzoic acid) in various pharmaceutical syrups and drops.

Keywords: Sequential injection analysis (SIA); Sequential injection chromatography (SIC); Monolithic columns; Ambroxol hydrochloride; Methylparaben; Benzoic acid

1. Introduction

The technique of sequential injection analysis (SIA) was described Ruzicka and Marshall for the first time at 1990 [1]. This analytical technique is based on forward, reversed, and stopped flow of the carrier stream and it has been the subject of hundreds scientific papers and several reviews [2–4].

The core of SIA system is a computer-controlled, multiposition valve, the centre port of which is connected via a holding coil to a high-precision syringe pump that is used to draw samples and reagents into the holding coil, to mix them in flow reverse mode and to transport the reacting mixture into the flow-through detector. Characteristic advantages of SIA thus include its versatility, full computer compatibility, high sample throughput, and low sample and reagent consumption.

About 14 years ago, Ruzicka proposed sequential injection technique as a new non-separation flow method. SIA determination of specific species in a matrix or mixtures was based on specific chemical conversion of analytes into detectable products using different detection methods. The majority of methods in speciation analysis currently still include a separation step before detection of different species. A recent literature survey revealed that high performance liquid chromatography (HPLC) is the dominant separation technique. Our innovative feature was that new hyphenated technique for analysis has been created coupling of short monolithic column with SIA manifold bringing a new dimension into SIA technique. The on-line coupling of the SIA technique with chromatographic monolithic column has been introduced in our previous works [5–8] and sequen-

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tial injection chromatography system (SIC) has been used for the determination of salicylic acid, methylsalicylate, methylparaben, propylparaben, paracetamol, caffeine, benzoic acid, sodium diclofenac and other compounds in different pharmaceutical preparations. However the designed SIC was used for another simultaneous determination of ambroxol hydrochloride (AM), benzoic acid (BA) and methylparaben (MP) in various pharmaceutical preparations (syrups and drops) in presented work.

Ambroxol, *trans*-4-(2-amino-3,5-dibromobenzylamino) cyclohexanol hydrochloride, is a pharmacologically active metabolite of bromhexine, and is used in pharmaceutical preparations as a mucolytic-expectorant agent due to its capacity to stimulate the transportation of the viscous secretion in the respiratory organs and reduces the standstillness of the secretion [9]. Ambroxol hydrochloride can be found in pharmaceutical preparations such as drops, granules, injections, syrups and tablets.

In most pharmaceutical preparations, especially in syrups, preservation is essential because the excipients, and sometimes the drug itself, may be destroyed by different microorganisms and consequently the formulation breaks down. Synthetic preservatives constitute the largest and most commonly used group in the preservation of pharmaceutical products. The esters of *p*-hydroxybenzoic acid with different alcohols, known as hydroxybenzoates or parabens, and benzoic acid are widely used as antimicrobial preservatives in liquid pharmaceutical forms [10].

Several different methods have been used for the individual determination of ambroxol hydrochloride in pharmaceutical preparations including LC–MS [11], spectrophotometry [12], HPLC [9,13,14], flow injection analysis [15,16], capillary electrophoresis and isotachophoresis [17,18]. More complex methods have been reported for ambroxol determination in biological fluids [17,19,20].

None of them combine the analysis of AM, BA and MP and only one of them [14] has been applied to a determination of ambroxol in presence of different preservatives. Nevertheless, chromatogram of the simultaneous determination of AM, BA and MP is not clearly presented in this paper and retention times mentioned in validation table show probable coelution of MP and BA.

Although MP and BA do not coexist in the same pharmaceutical preparation, their simultaneous separation and determination together with AM reflect the potential of the developed method and makes it most suitable for screen testing.

2. Experimental

2.1. Reagents

The standards of ambroxol hydrochloride and methylparaben were obtained from Sigma–Aldrich (Prague, Czech Republic), benzoic acid and internal standard salicylic acid (SA) were from Balex a.s. (Pardubice, Czech Republic). Stock standard solutions were prepared in methanol in concentration 1000 $\mu g\,ml^{-1}$ and were stored at −20 °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.

Methanol, acetonitrile and tetrahydrofuran (Chromasolv, for LC) were obtained from Sigma–Aldrich, acetic acid (98%) was from Lachema (Neratovice, Czech Republic) and triethylamine was from Sigma–Aldrich. All other chemicals used were of analytical grade quality. Tested pharmaceutical syrups and drops were: HALIXOL® syrup (Egis Pharm., Hungary), MUCOSOLVAN® syrup (Boehringer Ingelheim, Germany), BRONCHOPRONT® syrup (Heinrich Mack, Germany), AMBROXOL AL® drops (Aliud Pharma, Germany), MUCOSIN® syrup (Zentiva, Czech Republic), MUCOSIN® drops (Zentiva, Czech Republic). The deionised water was purified by a Milli-Q system (Millipore Corp., Bedford, MA).

2.2. Apparatus

A FIAlab® 3000 system (FIAlab® Instruments, USA) is a commercially produced instrument consisting of a syringe pump (syringe reservoir 5 ml) and six-port selection Cheminert valve (Valco Instrument Co., 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 solarization resistant optic fibers and 10 mm Z-flow cell were from Avantes Inc. (Colorado, USA). The whole SIA system was controlled by program FIAlab for Windows 5.0. Flow connecting 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® SpeedROD RP-18e, 50-4.6 mm column (Merck, Germany) with 10 mm monolithic precolumn. The monolithic column was placed between the six-port selection valve and flow cell of the detector. The mobile phase was aspirated through the filter ending (10 μm).

The comparative HPLC system, consisting of a binary pump LCP 4100 (Ecom, Prague), Waters autosampler 717 plus, variable wavelength UV detector Waters 486 (Waters, Milford, MA) and a PC for data processing, was controlled by chromatographic software CSW v.1.7 for Windows (Data Apex s.r.o., Prague). Analyses were performed on the same column.

2.3. Method and sample preparation

2.3.1. Mobile phase

The optimal mobile phase for separation of AM, BA, MP and internal standard SA was acetonitrile–tetrahydrofuran–0.05 M acetic acid (10:10:90, v/v/v), pH 3.75 adjusted with triethylamine. Mobile phase was degassed before application by means of helium.

The finally selected optimised conditions were as follows: injection volume 25 μl for standard solutions or sample of syrups and drops, the isocratic mobile phase was pumped at flow rate 0.48 $ml\,min^{-1}$ at ambient temperature, and the detection wavelength was 245 nm.

2.3.2. Solutions and sample preparation

Different commercial pharmaceutical syrups and drops were analysed such as HALIXOL® syrup, MUCOSOLVAN® syrup, BRONCHOPRONT® syrup, MUCOSIN® syrup (all of them contained 3 mg of ambroxol hydrochloride in 1 ml of syrup), and AMBROXOL AL® drops and MUCOSIN® drops (both contained 7.5 mg of ambroxol hydrochloride in 1 ml of drops). The content of preservatives (benzoic acid or methylparaben) was not specified and none pharmaceutical preparation contained combination of both of them.

Determination of the active substances and preservatives in the syrups and drops was done by the following procedure. Twenty microliters of syrup or 10 μl of drops were transferred to the 1 ml vial and 980 or 990 μl of internal standard SA solution in mobile phase in concentration 40 μg ml^{-1} was added. The samples were homogenized by 3 min sonication. The comparative standard solution for syrup or drops analysis was created by the same way: 60 or 75 μl of ambroxol hydrochloride stock standard solution were transferred to the 1 ml vial, different amount of stock solution of BA or MP was added according the composition of the pharmaceutical preparations, and total volume 1000 μl was obtained filling in internal standard SA solution in mobile phase in concentration 40 μg ml^{-1}. Unknown concentrations of the preservatives in pharmaceutical preparations were found by means of spiked standard additions.

A volume 25 μl of prepared sample was analysed by SIC system. Identification of peaks in the syrups and drops was based on comparison of the retention times of the compounds in standard solutions. 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 optimisation

Strategy of the compounds separation in this study consisted of three main points: to propose a sequential injection chromatography system with suitable column that ensures separation of all compounds of our interest, to find suitable mobile phase and pH conditions that enable optimal separation with high resolution; free of interferences from the matrix and excipients of syrups and drops. The last point was to show the possibility of this method for separation and analysis of real pharmaceutical sample without using the HPLC instrumentation. The main aim of the presented work has been focused on the optimisation of the SIC conditions with respect to the simple and fast, as well as low cost separation analysis of desired compounds.

The selection of an appropriate internal standard was made from compounds of similar structure as mentioned preservatives (*ortho*-chlorobenzoic acid, salicylic acid, paracetamol, *para*-aminobenzoic acid and caffeine). The last three compounds proved to have retention times that would interfere with ambroxol and were therefore ruled out. The chromatographic behaviour of *ortho*-chlorobenzoic acid and salicylic acid was similar, both of them did not interfere with the peak of the analytes and did not prolong time of the analysis. Salicylic acid was chosen as a suitable internal standard under the conditions of optimal mobile phase.

To our best knowledge no work dealt with simultaneous determination of AM, BA and MP. The work of Koundourellis et al. [14] demonstrates retention behaviour of ambroxol and parabens as a function of pH and methanol–water mobile phase composition. The work of Heinanen and Barbas [9] solves relationships between pH, acetonitril or tetrahydrofuran in mobile phase and retention times and peak symmetry of ambroxol and benzoic acid within reverse phase chromatography mode.

In reverse phase chromatography process, ionisation of the drug enhances the elution ability of mobile phase at low pH value for bases and at high pH value for acidic compounds. Ambroxol – basic compound (pK_{b1} = 1.39, pK_{b2} = 7.16), benzoic acid (pK_a = 4.19) and methylparaben–weak acid (pK_a = 8.30) are very different compounds from the acid–base standpoint and due to this aspect their reverse phase chromatography separation showed some troubles. Owing to acid–base characteristics of the compounds herein analysed, separation is highly dependent of pH, so the effect of this variable has been studied too. Three different C-18 monolith column lengths, four different organic phases (methanol, isopropanol, acetonitrile and tetrahydrofuran) were assayed as well as different strategies to avoid tailing in peaks: changes in pH of mobile phase or addition of "silanol blockers" (triethylamine). The optimisation was started with Chromolith® Flash RP-18 25 mm × 4.6 mm column with 10 mm precolumn. Mobile phase acetonitrile–phosphate buffer 20:80 (v/v), pH 4.0 adjusted with triethylamine, was tried as the initial experiment. The optimisation procedure continued by changes in ratios of acetonitrile, methanol or isopropanol and the changes pH of the mobile phase as well as combining of organic parts. Variation in the pH of mobile phases in the range 2.0–6.5 strongly affected retention behaviour of analytes and resulted in the change of elution order. Nevertheless, none of these modifications were good enough to get adequate resolution between the target analytes. The same results of insufficient resolution were achieved with the Chromolith® SpeedROD RP-18e, 50-4.6 mm column. Therefore the separation length of Chromolith® SpeedROD RP-18e, 50-4.6 mm column was enlarged with 10 mm monolithic precolumn as maximum column length usable for low-pressure syringe pump of SIC system without avoiding the stopping or damage of the glass syringe pump.

In order to achieve satisfactory results for the separation and quantification of compounds, together with a short time of analysis on the Chromolith® SpeedROD RP-18e, 50-4.6 mm column with 10 mm precolumn, next experiments were performed, variation in the organic part of the mobile phase, its proportions in relation to water (buffer) phase, and changing the pH. The flow rate 0.5 ml min^{-1} was not possible to exceed, because the system could not bear greater flow rates under these conditions, risking high back-pressure, that could stop the system. Therefore the flow rate chosen was 0.48 ml min^{-1}.

On the basis of the chromatographic behaviour of the compounds on the Chromolith® Flash RP-18 25 mm × 4.6 mm, find-

ing the next optimal conditions on the Chromolith® SpeedROD RP-18e, 50-4.6 mm column with 10 mm precolumn was simplified. At acid pH 2.5, where ionisation of BA and SA is considerably reduced, and ionisation of AM is complete, weak retention of AM (retention time close to solvent peak) and sufficient retention of MP and BA were observed. At neutral pH 6.0 where ionisation of AM is partially reduced, and ionisation of BA is almost complete, BA was eluted with solvent peak and strong retention (around 15 min) and broadness of AM was observed. Retention behaviour of MP under the mentioned conditions was not influenced (retention time was in the range 3–4 min). The next experiments with pH of mobile phases showed that optimum pH range was found to be between the values 3.50–4.50 pH units depending on the composition of organic part of the mobile phase. Acetic acid 0.05 M with addition of "silanol blocker" triethylamine was chosen as suitable organic buffer that was enabled to suppress peak tailing of ambroxol. The optimal pH of the mobile phase was determined as a value 3.75 ± 0.10 pH unit. This pH range had to be very strictly kept to avoid decreasing of peaks resolution or peaks coelution. Another subject to consider was the organic solvent employed in the mobile phase. The tested mobile phases were in the range 10–30% (v/v) of organic part in 0.05 M acetic acid–triethylamine buffer solution. The presence of methanol or isopropanol in the mixture showed a strong negative influence on peak widths. Better results were achieved using acetonitril-buffer 20:80 (v/v), however sufficient resolution of the peaks to reach the base-line was not observed—peaks of AM and BA were partially coeluted. The optimisation was continued changing 10% of tetrahydrofuran instead of 10% acetonitril in mobile phase and amount of buffer part was increased. This last step successfully affected selectivity of the mobile phase and resolution to the base-line was achieved between peaks of all compounds.

Final conditions for which the method was validated were: Chromolith® SpeedROD RP-18e, 50-4.6 mm column with 10 mm precolumn and acetonitrile–tetrahydrofuran–0.05 M acetic acid (10:10:90, v/v/v), pH 3.75 adjusted with triethylamine, flow rate 0.48 ml min^{-1}. Under these conditions the peak of internal standard—SA was eluted as the first peak and did not prolong time of the analysis. Proposed system enabled successful separation and sufficient retention of the target analytes was achieved. Representative sequential injection chromatogram showing successful separation of all compounds of interest is shown in Fig. 1. Long retention times of the analytes prolong time of the analysis, however the space of 150 s on the chromatogram between solvent peak and first peak of SA remains free for elution of polar interferences from pharmaceutical preparations as are sorbitol, propylenglycol, citric acid, glycerol or saccharine. The total mobile phase volume for one analysis was less than 5.0 ml and the time required less than 11 min.

From the UV spectra of all the analysed compounds in mobile phase, Fig. 2, the optimal detection wavelength of 245 nm was chosen providing compromise between the absorption maximum of compounds. Peak height evaluation was performed using the FIAlab® software.

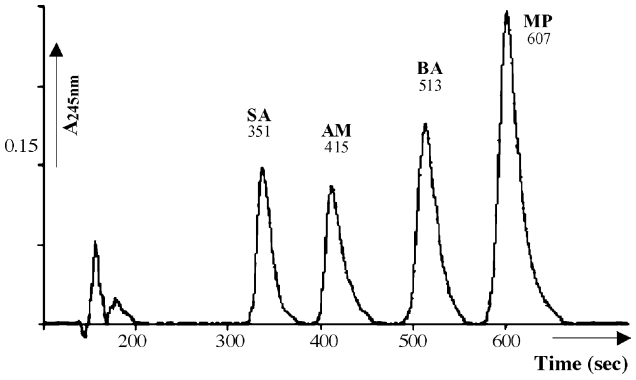

Fig. 1. SI chromatogram of the separation of the compounds included in expectorant pharmaceutical preparations. Mobile phase: acetonitrile–tetrahydrofuran–0.05 M acetic acid (10:10:90, v/v), pH 3.75 adjusted with triethylamine, flow rate 0.48 ml min^{-1}, UV detection at 245 nm.

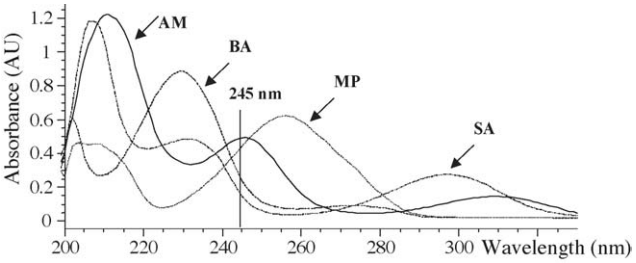

Fig. 2. Spectra of the analysed compounds in mobile phase: ambroxol (AM), benzoic acid (BA), methylparaben (MP) and internal standard salicylic acid (SA).

3.2. Parameters of sequential injection chromatography process

The desired compounds were successfully separated using the proposed procedure and basic chromatographic parameters were calculated from experimental data, such as peak symmetry factor, resolution factor, number of theoretical plates and they are given in Table 1.

The parameters characterising the robustness of the method (its ability to remain unaffected by small changes in the parameters such as organic modifier content and pH of the mobile phase, buffer concentration, temperature, and injection volume) were discussed. Due to limited length of the column the maximum content of organic part was 20% (v/v) of acetonitrile or tetrahydrofurane together in mobile phase. Total content of organic part higher than 20% (v/v) resulted in peaks coelution. Lower contents of organic part, less than 15% (v/v) caused increasing

Table 1
The parameters of SIC process

	SA (i.s.)	AM	BA	MP
Retention time (s)	351	415	513	607
Peak resolution	$R_{SA,AM}$ = 1.70	$R_{AM,BA}$ = 1.89	$R_{BA,MP}$ = 1.74	
Peak symmetry	1.45	1.47	1.62	1.37
Number of theoretical plates	1706	2380	3120	2652

of peak width and rising volume of mobile phase that is limiting due to the volume of the syringe. Owing to the different acid–base characteristics of the compounds, optimisation of the mobile phase pH was one of the important conditions for the separation. The optimum pH for successful separation was in the range 3.75 ± 0.10. This pH range had to be very strictly kept to avoid decreasing of peaks resolution or peaks coelution. The resolution between the peaks SA, AM and BA was suddenly decreasing and change of the elution order could be observed at pH values out of this range.

3.3. Analytical parameters and validation

The optimised method was validated in order to evaluate if adequate linearity, sensitivity, repeatability, recovery, selectivity, precision and accuracy had been achieved.

Linearity was established with a series of working solutions prepared by diluting the stock solution in mobile phase to the final concentrations. Each concentration was injected in triplicate and the mean value of peak height was taken for the calibration curve. The calibration graphs involved at least seven experimental points for each compound and they are described by the following equations: for AM: $A = (0.002356 \pm 0.000099)c + (0.012341 \pm 0.004367)$ (where A is the absorbance and c the analyte concentration), the correlation coefficient was 0.9957; for BA: $A = (0.005201 \pm 0.000078)c - (0.000956 \pm 0.003436)$, the correlation coefficient was 0.9994; for MP: $A = (0.011131 \pm 0.000289)c - (0.015174 \pm 0.006952)$, the correlation coefficient was 0.9986; for internal standard SA: $A = (0.004287 \pm 0.000061)c - (0.003955 \pm 0.001465)$, the correlation coefficient was 0.9996.

The limit of detection (LOD) was calculated by comparison of the three-fold variation of signal to noise ratio (3S/N) obtained from analysis of the blank sample, 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 (10S/N).

The intra-day precision of the method was determined by preparing the standards of AM, BA, MP and SA at three concentration levels and peak heights for each compound were determined after processing each standard seven times. The inter-day repeatability was followed at the middle concentration level for three consecutive days. The results are summarized in form of R.S.D. The method validation results obtained under the final conditions are shown in Table 2.

The method was found to fulfil common requirements of accuracy, precision and linearity (calibration range with correlation >0.995, R.S.D. for repeated standard injections at three concentration levels ($n = 7$) less than 4.5%). Correlation with the HPLC determination (t-test) has been achieved.

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 analysed consecutively. This approach provides a means of covering the precision of the entire method, from sample preparation to data handling. The accuracy of the method was carried out measuring of the samples fortified with known quantity of the analytes. The procedure was made under the following conditions: 20 μl of syrup or 10 μl of drops were transferred to the 1 ml vial and than spiked with known quantity of standard solutions to reach for two-fold concentration, and internal standard SA solution in mobile phase in concentration 40 μg ml^{-1} was added to the final volume 1000 μl. The samples were homogenized by 3 min sonication. The comparative solutions of syrup or drops were created by the same way: 20 μl of syrup or 10 μl of drops were transferred to the 1ml vial, and total volume 1000 μl was obtained filling in internal standard SA solution in mobile phase in concentration 40 μg ml^{-1}. Spiked sample solutions and un-spiked sample solutions were compared for recovery evaluation. The method accuracy results are shown in Table 3 and values of the

Table 2
Analytical parameters and method validation results

	SA (i.s.)	AM	BA	MP
Calibration range (μg ml^{-1})[a]	2–50	2–100	2–100	2–50
Correlation coefficient	0.9996	0.9957	0.9994	0.9986
Limit of detection (μg ml^{-1})	2	1	1	2
Limit of quantification (μg ml^{-1})	6.7	3.3	3.3	6.7
System precision (%)[b]				
100 μg ml^{-1}	3.13	0,89	3.59	4.44
50 μg ml^{-1}	2.18	2.26	1.93	1.55
LOQ	2.64	1.87	2.87	1.91
System precision (%)[c] 50 μg ml^{-1}	2.46	3.17	3.75	2.41
Repeatability of T_r (%)[d]	0.25	0.61	0.29	0.38
	1.56	1.80	2.67	3.40

[a] Each concentration was measured in triplicate.
[b] Intra-day relative standard deviation (R.S.D.) values were calculated for repeated standard injections at three concentration levels $c = 100$ (μg ml^{-1}), $c = 50$ (μg ml^{-1}) and $c =$ LOQ; $n = 7$.
[c] Inter-day R.S.D. values were calculated for repeated standard injections at concentration level $c = 50$ (μg ml^{-1}); $n = 7$; three consecutive days.
[d] Repeatability of T_r—R.S.D. of retention times for intra-day (and inter-day–bottom line) repeated standard injections; $n = 7$; (three consecutive days).

Table 3
The results of the recovery of the method for pharmaceutical preparations (syrups, drops)

Pharmaceutical preparation (syrup, drops)	Composition	Added amount (%)	Found amount (%) ±R.S.D. from added amount
Bronchopront syrup	AM	100	101.5 ± 5.3
	MP	100	99.4 ± 7.4
Halixol syrup	AM	100	99.1 ± 5.8
	BA (sodium benzoate)	100	94.1 ± 1.2
Mucosolvan syrup	AM	100	100.9 ± 2.2
	BA	100	95,6 ± 5.6
Ambroxol Al drops	AM	100	99.9 ± 4.2
	BA	100	95.0 ± 2.6
Mucosin with honey drops	AM	100	98.5 ± 2.3
	MP	100	105.8 ± 4.4
Mucosin with honey syrup	AM	100	100.9 ± 2.2
	BA (sodium benzoate)	100	98.8 ± 4.4

Table 4
Determination of ambroxol hydrochloride, methylparaben and benzoic acid in various syrups and drops

Pharmaceutical preparation (syrup, drops)	Composition	Found amount in (%) ±R.S.D.	
		SIC	HPLC
Bronchopront syrup	AM	104.2 ± 1.3	104.5 ± 2.1
	MP	98.5 ± 4.3	99.3 ± 1.9
Halixol syrup	AM	103.2 ± 3.4	101.5 ± 2.3
	BA (sodium benzoate)	100.0 ± 4.0	99.2 ± 3.1
Mucosolvan syrup	AM	100.1 ± 2.3	98.3 ± 2.4
	BA	101.0 ± 1.9	103.2 ± 3.4
Ambroxol Al drops	AM	102.1 ± 2.4	101.9 ± 1.6
	BA	96.1 ± 1.7	98.2 ± 1.2
Mucosin with honey drops	AM	97.3 ± 2.2	99.1 ± 2.9
	MP	101.1 ± 3.6	102.5 ± 3.2
Mucosin with honey syrup	AM	104.8 ± 1.8	104.3 ± 2.2
	BA (sodium benzoate)	97.8 ± 4.4	98.4 ± 3.0

Results obtained SIC and HPLC method.

recoveries were found in the range 94.1–105.8%. Assay values of recoveries show that the method allows direct determination of ambroxol and preservatives in commercial dosage forms in the presence of other excipients. In syrups, saccharine, sorbitol, propylenglycol, citric acid and glycerol are eluted very near or inside the solvent peak due to their high polarity.

3.4. Determination in various expectorant syrups and drops

The method developed has been applied to the determination of AM, BA and MP in different expectorant syrups and drops with high recovery values. The samples were commercially available on the Czech market. The interference effect of excipients (saccharine, sorbitol, propylenglycol, citric acid, glycerol and essence honey) was not observed. The optimal extraction medium for syrups or drops dissolution was mobile phase. The procedure of sample preparation was simple, fast and achieving high precision and low sample and reagent consumption. The found average amounts of AM, BA and MP of the labelled amount in the syrups and drops are given in Table 4. The results were compared with those obtained by HPLC method under the same chromatographic conditions. The statistical *t*-test (95% level) revealed no significant difference between the average values found by both methods. Both results are in a good agreement with the pharmacopoeial requirements on the active compound content in the pharmaceutical syrups and drops.

4. Conclusion

The results presented provide an evidence that the hyphenated SIC method – combination of SIA technique with monolithic column – can be an alternative to other frequently applied techniques for sample separation, such as HPLC or CE technique. The method has been applied with satisfactory precision and accuracy to the determination of AM, BA, MP in various types of the pharmaceutical expectorant preparations. The method is acceptably time efficient; a single analysis takes about 11 min. Compared with earlier published LC methods utilised for the determination of AM, the proposed assay shows somewhat lower sensitivity but it is still fully sufficient for the analysis of pharmaceutical preparations containing AM and BA, or MP as the preservatives. The possibility of determining milligram amounts of AM, BA or MP in pharmaceutical preparations and low running costs make the SIC method a good alternative to existing methods currently used in quality control of various pharmaceutical dosage forms.

Nowadays, the monolithic columns are very popular on the field of chromatography.

Their insertion in sequential injection systems offers interesting features, such as reagent economy, flow simplicity, on-line sample pre-treatment, and separation without expensive HPLC instrumentation. The SIC technique opens a new and important field in pharmaceutical analysis for the simultaneous assay of compounds in combined pharmaceutical preparations. Nevertheless the main disadvantage of the system is persisting limited operating back-pressure of the system. On the other hand, SIC method could fill the gap between the traditional HPLC and low-pressure no-separation flow method such as flow injection and sequential injection analysis.

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