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Flow-injection spectrophotometric determination of salicylate using on-line solid phase extraction

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ABSTRACT

In the proposed procedure determination of salicylate using a solid phase extraction (SPE) integrated to a flow-injection FIA system is described. This enables to use biological samples (urine, blood serum) for analysis without pretreatment, to concentrate an analyte and to increase the selectivity of determination by the removal of interfering substances. With the use of the SPE column (quaternary ammonium), salicylic acid, in the range from 0.05 to 20 μg/ml, RSD=0.44%, with frequency 30 to 60 samples in an hour, was determined.

INTRODUCTION

The solid phase extraction (SPE) became the very popular technique for separation and preconcentration of inorganic and organic substances especially with the analysis of biological samples. In biochemical and pharmaceutical laboratories the SPE is used for the isolation of effective substances from biological liquids. In bioanalysis the isolation of drugs before the determination serves mainly for their preconcentration and removal of a number of endogenous undesirable substances. This technique,largely utilized for the preparation of samples before an analysis, increases specification and sensitivity of analytical method used. Its off-line performance is both time consuming and complicated and thus it is usually the slowest step of the analysis. One of the possibilities how to facilitate and accelerate the analysis of samples is to integrate the mentioned preparation of samples with the use of the SPE directly to the flow-injection system of FIA analyser. Therefore real biological samples, without any pretreatment, can be directly applied to the flow-injection system.

EXPERIMENTAL

Reagents

The solution of a reagent: 20,2 g $Fe(NO_3)_3.9H_2O$ was dissolved in 1000 ml of 0,02 mol/l nitric acid. All other chemicals were of analytical-reagent grade. Deionized distilled water was used throughout for the preparation of solutions and as a carrier stream. A stock solution of salicylic acid was prepared by dissolving of weight in water and working standard solutions were prepared by appropriate dilution of the stock standard solution to give final concentrations of 0,05 to 20 μg/ml salicylic acid. All solutions were filtered through a 0,85 μm filter and degassed under reduced pressure.

Manifold and apparatus

The flow-injection manifold is shown in Fig.1. It consists of a commercial FIA-20 Analyser which is linked to a Spekol 10 single-beam photometer (Zeis, Jena, Germany) equipped with a EKMi accesory consisting of a flow-through cell of a path length 30 mm, a logarithmic convertor (laboratory made) and a TZ 4600 chart recorder (Laboratorní přístroje, Prague, Czech Republic). All connections and reaction coils were made using a 0,5 mm i.d. PTFE tubing.

Procedure

With the turning of an injection valve to the position A (Fig.1), the given volume of a sample was injected into the flow-injection system and an analyte was adsorbed on a sorbent of the column, the column was washed with the carrier stream and consequently a loop of the injection valve was filled with an eluting solvent. In the position B, the given volume of the eluting solvent was lead to the column to elute the analyte and the column was washed with the carrier stream and at the same time a next sample was filled into the loop of the injection valve.

SPE column

For the preparation of the column, silica gel modified with quaternary ammonium (Baker) was packed in a PTFE tubing with 2 mm i.d. and 3 to 5 mm long

and the both ends were closed with glass wool. This prepared SPE column was placed instead of one injection loop of the valve (Fig. 1).

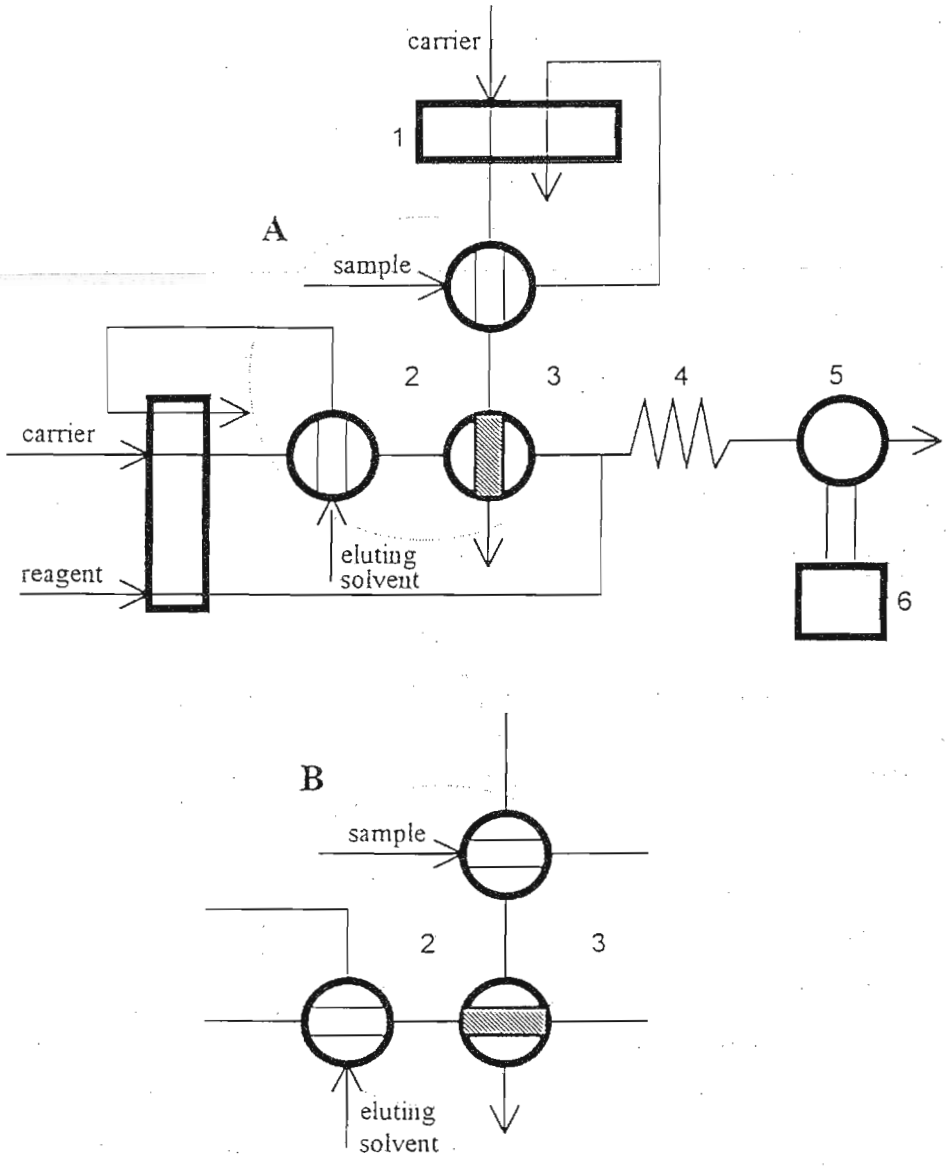

Fig. 1: Schematic diagram of the manifold for salicylate determination
1- peristaltic pumps, 2- injection valve, 3- SPE column, 4- reaction coil,
5- detector, 6- chart recorder;
A - injection and sorption of sample, B - injection of eluting solvent

RESULTS AND DISCUSSION

Optimization of analytical parameters

Optimal conditions found for the determination of salicylate with the use of the flow-injection system with the integrated SPE column are shown in Table 1. At the same time requirements on the SPE column i.e. sufficiently large capacity and full elution of an adsorbed sample after an addition of an eluting solvent were best fulfilled for the column with the quaternary ammonium modified silica gel.

Table 1: Optimum values of variables of FIA system for the determination of salicylate

Variable	Unit	Values
Injection volume of sample	µl	40 to 500
Coil length	cm	50
Reagent $Fe(NO_3)_3$	g/l	20,2
in HNO_3	mol/l	0,02
flow-rate	ml/min	0,25
Carrier H_2O flow-rate	ml/min	0,55
Eluent HNO_3	mol/l	0,05
Injection volume of eluent	µl	50
Flow cell (light pass length)	mm	30
Wavelength	nm	540

Under the mentioned conditions fiagram for standard aqueous solutions of salicylic acid was shown in Fig. 2. Calibration curve obtained from the peaks' height in the absorbance units (an average from three values) is linear for solutions in the concentration range from 1,0 to 20 µg/ml of salicylic acid (injected volume 200 µl) and is characterized by equation of linear regresion $A = 0,03671.c - 0,0355$; correlation coefficient $r = 0,99973$.

Reproducibility of the determination for a salicylic acid solution of 10µg/ml is characterized by relative standard deviation RSD = 0,44 % for n = 10. Also the dependence of bonded salicylate on the volume of injected solution in the range from 40 to 500 µl with the content of 4 µg/ml of salicylic acid is linear. It gives the evidence

about that under the given conditions with the injection of a sample, the full retention of salicylates from the sample takes place and by an affect of the eluting solvent, the full elution from the column takes place. This enables to determine solutions of the concentration of 0,05 μg/ml of salicylic acid by injecting of 500 μl of the sample.

An average capacity of the column is 45 mg of salicylic acid per 1 gram of a dry sorbent and this means that the capacity of the column used is at least 100 times higher than the amount of salicylate in the most concentrated calibration solution.

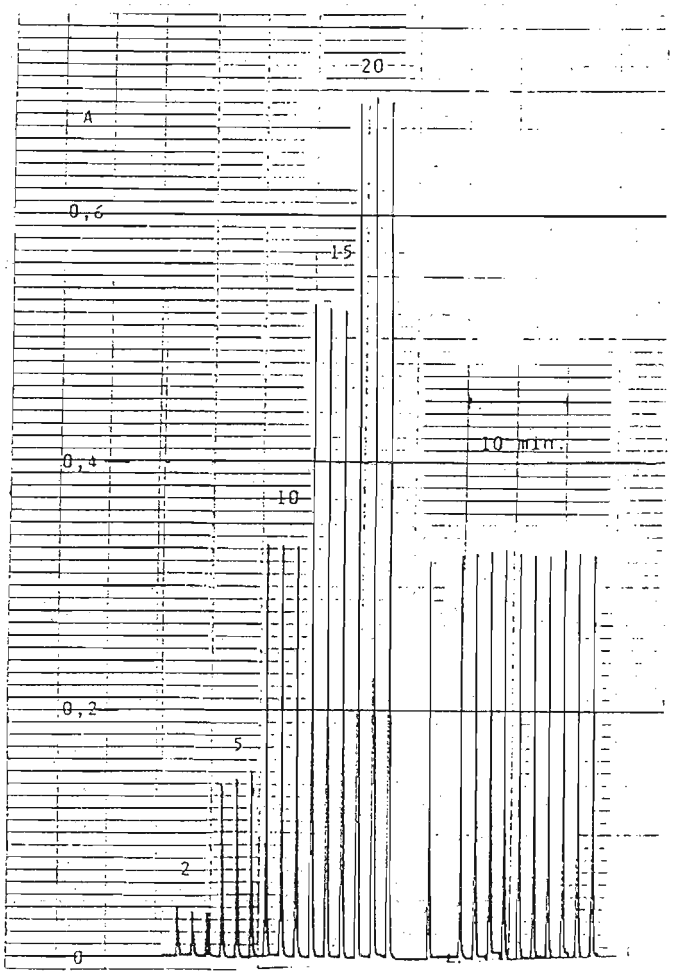

Fig. 2: Fiagram of standard solutions of salicylic acid 2 - 20 μg/ml
Reproducibility for 10 μg/ml

Interference studies

Spectrophotometric determination of salicylic acid is negatively affected by the presence of compounds, which give colourless complexes with ferric ions such as phosphates and oxalates. A high concentration of the reagent - ferric nitrate was chosen for the removal of the mentioned influence. All compounds, which give coloured products with ferric ions also interfere the determination. Here the advantages of the use of the SPE column in the flow-injection system were shown. The SPE column under the given conditions retains only anions, but the other compounds are not retained and are eluted out with the carrier stream. Only the retained anions are then released from the column by the nitric acid elution to the flow-injection system.

Compounds, which expressively interfere the determination of salicylic acid without the use of the SPE column [1] are shown in the Table 2. With the use of the SPE column they do not interfere the determination or their influence is substantively decreased.

Table 2: Influence of interfering substances on the determination of salicylic acid

Interferent	Without SPE		With SPE	
	Addition µg/ml	found (1) %	Addition µg/ml	found (this work) %
Salicylic acid	25	100,0	25	100,0
Phenol	25	105,7	100	100,0
p-Aminophenol	25	109,2	50	100,0
o-Aminophenol	25	188,0	25	100,0
4-Aminophenazone	25	132,6	100	100,0
Oxytetracycline	25	128,8	50	100,0

Salicylic acid 25 µg/ml

Determination of salicylic acid in urine and blood serum

For the determination of salicylate in urine the sample is diluted 5 to 20 times with water and directly is used for the determination. As it is shown in Table 3, any physiological element of urine nor oxalates do not interfere the determination [2].

Also for the determination of the content of salicylate in blood serum the given sample is diluted 5 or 10 times with water and is directly injected to the analyser without any other treatment. The results given in Table 3 indicate that the recoveries are almost quantitative. The method with the use of the SPE column is at the same time more sensitive and more selective [3, 4].

Table 3: Determination of salicylate in urine and serum samples

	Salicylate added µg/ml	Found[a] µg/ml	Recovery %
Urine[b]	50	50,3	100,6
	100	98,0	98,0
	200	197,0	98,5
Urine[c]	10	9,9	99,0
	50	50,7	101,4
	100	98,5	98,5
Serum[d]	10	10,2	102,0
	20	19,6	98,0
	50	49,3	98,6
Serum[e]	0	8,2	-
	10	18,3	100,5
	20	27,9	98,9

[a] Mean of 3 measurements
[b] Sample diluted 20 times
[c] Sample diluted 5 times
[d] Standard serum Lyonorm U diluted 5 times
[e] Sample diluted 5 times

CONCLUSIONS

The mentioned results indicate that the SPE column (2 mm i.d., filling length 10 to 30 mm of solid phase quarternary ammonium) is possible to use for the determination of salicylic acid in the flow-injection FIA analyser repeatedly many times. With injection of larger volume of the sample it is possible to preconcentrate an analyte and by that to increase the sensitivity of the determination. The SPE column, however, serves mainly for the removing of the interfering elements during the determination in biological liquids and therefore for increasing the selectivity of the determination. At the same time the frequency of injections of samples (30 to 60 per hour) is not expressively decreased.

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