High-Performance Liquid Chromatography Analysis of Thiazolidine-4-carboxylic Acids in Human Serum Samples

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In the presented paper we studied analytical characteristics of three thiazolidine-4-carboxylic acids. These compounds obtained by condensation of aldehydes with L-cysteine are interesting from the biological point of view. The proposed method involves a solid phase extraction (SPE) of thiazolidinic acids and their precursor aldehydes from human serum using C_{18} SPE columns. Separation of the six analytes was achieved within 20 min using a reversed-phase HPLC SEPARON SGX C_{18} column protected by precolumn, with mobile phase of methanol—water (φ_r = 55 : 45) containing 5 cm^3 of acetic acid per 1 dm^3 of mobile phase (apparent pH 5.5). The recoveries of all studied compounds from the human serum were in the range of 86—89 %, the detection limits were 30 ng cm^{-3} of serum.

Thiazolidine compounds are formed by condensation of either aliphatic or aromatic moieties, containing a CHO group, with different aminothiols [1, 2]. We obtained several thiazolidine-4-carboxylic acids – TAs (Scheme 1) by condensation of carbonyl derivatives and L-cysteine according to the published procedures [3—5].

Aromatic thiazolidine compounds could have physiological importance. The thiazolidine-4-carboxylic acids are known to be nontoxic compounds and they even show a broad spectrum of significant biological activities [6—8]. It should be noted that the thiazolidine ring is a building unit of penicillin antibiotics. Some authors examined the ability of this ligand structure to form complexes with some radionuclides for potential use in nuclear medicine [9, 10]. The spectrophotometric procedure of *Guidotti et al.* [11] is very useful for the determination of the aliphatic TAs but this method is unsuitable for aromatic derivatives. Recently some authors published high-performance liquid chromatographic (HPLC) procedures for the separation of thiazolidinic derivatives. *Sen et al.* [12] reports the separation of selected thiazolidinic derivatives found in the food type of samples. *Van Doorn et al.* [13], *Ogata* and *Taguchi* [14] developed HPLC methods for the determination of urinary 2-thiothiazolidine-4-carboxylic acid as an index of carbon disulfide exposure.

Since the thiazolidine compounds are easily formed under physiological conditions (pH 7 and 37°C), it is likely that they are also formed *in vivo*. Therefore, it is important to investigate their biological role. To solve this problem, the simple and reliable procedure was needed for the isolation and determination of TAs from biological matrix. The presented paper describes the simple and rapid isolation and separation procedure for three thiazolidine-4-carboxylic acids from human serum.

EXPERIMENTAL

Compounds *I*—*III* were synthesized from benzaldehyde (*AI*), 2-hydroxybenzaldehyde (*AII*), and 4-chlorobenzaldehyde (*AIII*) with L-cysteine, by known methods [3—5]. Their purities were tested by elemental analysis, IR and NMR spectra.

Acetic acid, NaOH, KH_2PO_4 were purchased from Lachema (Brno, Czech Republic), H_2O for preparation of the mobile phase was double-distilled, CH_3OH (HPLC grade) Lab-Scan (Dublin, Ireland) was used.

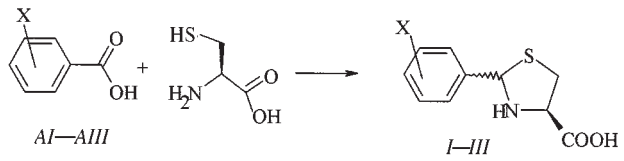
Aqueous solution of the compounds showed a specific UV spectrum with absorption maxima at $\lambda = 280$ nm with $\varepsilon/(dm^3\ mmol^{-1}\ cm^{-1})$: 8.2 (*I*), 9.6 (*II*), 7.9 (*III*). The spectra were obtained using a spectrophotometer Specord M 40 (Zeiss, Jena).

Stock solutions were prepared by adding 10 mg of substances to 100 cm^3 of mobile phase. The stock solutions were serially diluted with mobile phase to give 100 μg cm^{-3} of each compound.

Human serum standards were prepared fresh daily by aliquoting appropriate amount of working solutions into 0.5 cm^3 of blank human serum to produce a concentration series ranging from 0.05 to 20 μg cm^{-3} of each compound.

Processing of Serum Samples

The C_{18} columns (100 mg, 1.0 cm^{-3}, Tessek,



AI, I X = H
AII, II X = 2-OH
AIII, III X = 4-Cl

Scheme 1

Formation of thiazolidine-4-carboxylic acids *I—III* by condensation of L-cysteine and benzaldehyde (*AI*), 2-hydroxybenzaldehyde (*AII*), and 4-chlorobenzaldehyde (*AIII*).

Prague, Czech Republic) placed on the vacuum extraction unit (Vac-Elut, Varian, City, Ca) were prewashed with 1 column volume of CH_3OH followed by 1 column of 0.1 M-KH_2PO_4 buffer solution. A 0.5 cm^3 aliquot of unknown sample or serum standard and 0.5 cm^3 of 0.1 M-KH_2PO_4 buffer solution were loaded onto individual SPE columns under slight vacuum. The column was rinsed with 0.3 cm^3 of methanol—0.1 M-KH_2PO_4 (φ_r = 20 : 80) followed by 1 cm^3 of 0.1 M-KH_2PO_4 buffer solution and then dried for 5 min with air by vacuum aspiration. The compounds of interest were eluted from the column with 2.0 cm^3 of methanol—water (φ_r = 55 : 45) by manually applying a slow uniform pressure to the top of the column. Each eluate was collected and 20 mm^3 of the eluate was injected onto the HPLC for analysis.

The HPLC system consisted of an Axiom-Chrom 717 integrator (Moorpark, California, USA) equipped with a UV-150 detector (Spectra Physics, USA), high-pressure L-6200 A intelligent pump (Merck, Germany). The chromatographic separation was carried out on a SEPARON SGX C_{18} reversed-phase column (150 mm × 3.3 mm I.D., 7 μm particle size, Tessek, Prague, Czech Republic) protected by precolumn, with mobile phase of methanol—water (φ_r = 55 : 45) containing 5 cm^3 of acetic acid per 1 dm^3 of mobile phase with an apparent pH 5.5 ± 0.05 adjusted with natrium hydroxide at the flow-rate of 0.5 cm^3 min^{-1} and the detection wavelength was 258 nm. The injection volume was 20 mm^3.

RESULTS AND DISCUSSION

We optimized the separation of TAs from the starting reagents (*AI, AII*, and *AIII*). The influence of organic solvent content in the mobile phase on the capacity factors of studied compounds is shown in Fig. 1. Fig. 2 shows a good separation obtained under isocratic conditions. Using the above eluent the capacity factors of separated compounds were in the range 3 to 7. This was satisfactory in terms of the interference of studied compounds with polar components from human serum eluting before studied TAs and aldehydes (Fig. 3). Under given conditions studied compounds

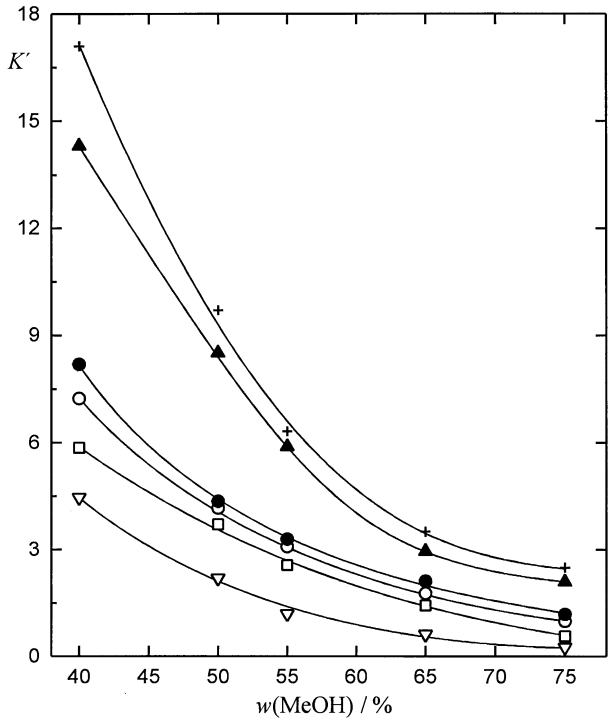


Fig. 1. The influence of methanol content in mobile phase on capacity factors. □ *I*, ○ *II*, ▲ *III*, ▽ *AI*, ● *AII*, + *AIII*.

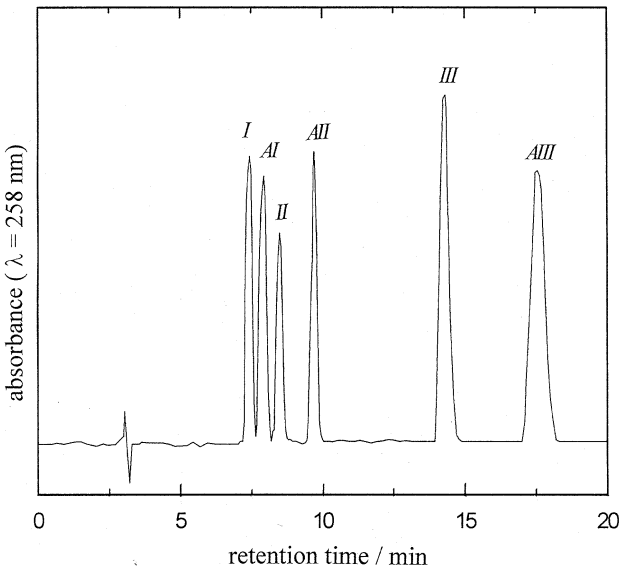


Fig. 2. Separation of compounds with detection at λ = 258 nm. Injection amount: 2 μg cm^{-3} of each standard in mobile phase. For chromatographic conditions see Experimental.

do not interfere with other biological species in serum affecting successful determination and HPLC separation. The free aminothiol (L-cysteine) does not interfere under our chromatographic conditions (UV detection mode at 258 nm).

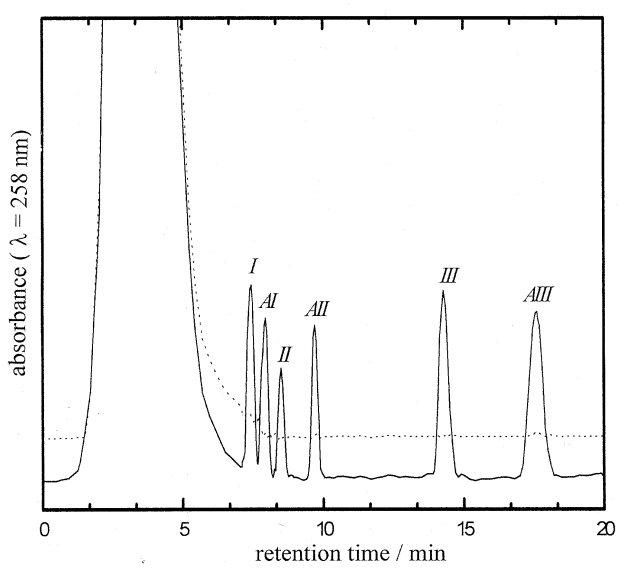


Fig. 3. The chromatogram of human serum samples: —— spiked with 2 μg of each compound per cm^3 of serum, ····· blank serum. For chromatographic conditions see Experimental.

Table 1. Correlation Coefficients r and Regression Equations. Concentration Range 0.05—20 μg cm^{-3} for *AI*, *AIII*, *I*, *III* and 0.1—30 μg cm^{-3} for *AII*, *II*

Compound	Regression equation	r
AI	$y = 1.45x - 0.14$	0.9999
AII	$y = 0.89x + 0.07$	0.9998
AIII	$y = 1.27x - 0.19$	0.9999
I	$y = 1.54x + 0.04$	0.9999
II	$y = 0.65x - 0.01$	0.9998
III	$y = 1.05x - 0.47$	0.9997

y – the peak area, x – the concentration.

Calibration curves for TAs *I* and *III*, *AI* and *AIII* in serum were linear in the range 0.05—20 μg cm^{-3} and for TAs *II*, *AII* in the range 0.1—30 μg cm^{-3}, respectively. The detection limits for the analyzed compounds, based on a signal-to-noise ratio of 3 were 30 ng cm^{-3} of human serum. Correlation coefficients were very close to the value of 1 and regression equations are reported in Table 1.

The recovery of studied compounds from human serum was examined in order to confirm effectivity

Table 2. The Recoveries of Studied Compounds from Human Serum with Different Final Concentration of Studied Compounds 0.1 μg cm^{-3}, 0.4 μg cm^{-3}, and 4 μg cm^{-3}

Compound	Recovery (± R.S.D., $n = 5$)/%		
	$\rho_1 = 0.1$ μg cm^{-3}	$\rho_2 = 0.4$ μg cm^{-3}	$\rho_3 = 4$ μg cm^{-3}
I	86.1 ± 1.6	86.4 ± 1.4	87.1 ± 1.5
II	86.5 ± 1.8	87.0 ± 1.2	88.0 ± 1.4
III	89.1 ± 1.8	89.3 ± 1.5	89.5 ± 1.3
AI	88.7 ± 1.8	89.0 ± 1.8	89.3 ± 1.6
AII	88.6 ± 1.8	89.1 ± 1.8	89.7 ± 1.4
AIII	88.3 ± 1.8	88.8 ± 1.8	89.2 ± 1.2

Table 3. The Reproducibilities of Retention Times and Peak Areas

Parameter	Compound	Retention time	S.D. ($n = 5$)	Relative S.D.	Peak area	S.D. ($n = 5$)	Relative S.D.
		min	min	%	a.u.	a.u.	%
Run-to-run reproducibility							
(within 1 day)	*I*	7.43	0.03	0.40	18.52	0.05	0.27
	II	7.95	0.01	0.23	17.23	0.06	0.35
	III	8.50	0.02	0.23	10.45	0.18	1.72
	AI	9.72	0.02	0.20	14.26	0.21	1.47
	AII	14.33	0.01	0.07	16.42	0.15	0.91
	AIII	17.62	0.03	0.17	20.18	0.04	0.20
Day-to-day reproducibility							
	I	7.41	0.05	0.67	18.42	0.23	1.25
	II	7.97	0.03	0.38	17.14	0.30	1.75
	III	8.53	0.04	0.49	10.39	0.54	5.20
	AI	9.71	0.06	0.62	14.15	0.65	4.60
	AII	14.36	0.04	0.28	17.28	0.85	4.92
	AIII	17.63	0.03	0.17	20.06	0.72	3.59

of SPE isolation step. Spiked samples were prepared by adding known amount of each compound to blank serum. The recoveries were calculated analyzing 3 serum samples with different final concentrations of studied compounds 0.1 μg cm^{-3}, 0.4 μg cm^{-3}, and 4 μg cm^{-3}. Five replicates were performed for each concentration studied. The recoveries found were higher than 86 % for all studied compounds under conditions described. The results are shown in Table 2.

The run-to-run and day-to-day reproducibilities of the retention times and peak areas were also studied. The reproducibilities of retention times calculated from 5 runs were in the range 0.07 to 0.40 % for run-to-run and 0.17 to 0.67 % for day-to-day experiments. The reproducibilities of peak areas calculated from 5 analyses were found in the intervals 0.2 to 1.72 % for run-to-run and 1.25 to 5.20 % for day-to-day experiments (Table 3).

CONCLUSION

Our proposed SPE/HPLC procedure is a simple, rapid, and reliable method for determination of studied TAs and their precursor aldehydes on low sub-ppm concentration level in human serum samples. The proposed method may be a very useful tool for studying the biological role and distribution of thiazolidinic acids and their precursor aldehydes in the *in vivo* systems.

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