Journal of Chromatography A, 660 (1994) 67–73
Elsevier Science B.V., Amsterdam

CHROMSYMP. 2945

Mobile phase selection for high-performance liquid chromatographic analysis of novel zinc(II) carboxylates with N-donor ligands

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ABSTRACT

The PRISMA optimization project was used for mobile phase selection in the HPLC analysis of novel zinc(II) carboxylates of the type $Zn(RCOO)_2 \cdot L_n \cdot q(H_2O)$ with N-donor ligands. The composition of the mobile phase is characterized by the solvent strength (S_T) and the selection points (P_S). At a constant S_T the correlation between P_S and retention data can be described by a quadratic function. For constant P_S the solvent strength and retention data correlate with a logarithmic function. On the basis of the results obtained in the TLC separation of the single zinc(II) carboxylate, solvents were chosen (ethanol, acetonitrile, methanol, dioxane, 2-propanol) that are suitable for HPLC analyses of zinc(II) carboxylates on LiChrosorb RP-8 and RP-18 columns.

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0021-9673/94/$07.00
SSDI 0021-9673(93)E0965-W

INTRODUCTION

Zinc(II) carboxylates of the type $Zn(RCOO)_2 \cdot L_n \cdot q(H_2O)$ (where R = H, CH_3, CH_3CH_2, $CH_3CH_2CH_2$, or $(CH_3)_2CH_2CH_2$, L = caffeine, thiourea, nicotinic acid or phenazone, $n = 1$ or 2 and $q = 0.5$, 1.5, 2.5, 3.5 and x is the variable content of water of crystallization) are an interesting group of substances. Nearest to them in pharmacological importance are zinc(II) compounds with pyrithione (1-hydroxypyridine-2-thione). Although the HPLC analysis of these substances is well known [1,2], there is very little information on the chromatographic analysis of novel zinc(II) carboxylates with N-donor ligands (their structure is unknown but is currently being investigated).

Successful tests of the biological activity of these newly synthesized compounds require analytical methods for the determination of zinc(II) cation, carboxylate anion, ligand metabolites and also non-metabolized forms of the original zinc(II) carboxylates. With regard to the nature of the compounds, HPLC [with comparative zinc(II) determination by atomic absorption spectrometry (AAS)] appears to be the most suitable method of analysis.

In the first phase of analysis, selection of the optimum mobile phase plays a most important role. Many studies have been based on the results of preliminary TLC or HPTLC analyses, the optimum composition of the mobile phase being chosen using the simplex method [3], statistical techniques [4] and multifactor analysis [5,6].

At present, optimization of mobile phase composition depends entirely on computer techniques and it is possible to select within a relatively short time the most suitable multicomponent mobile phase [7–13] or a buffer mixture [14], and it is also possible to identify individual peaks [15–17], in addition to the selection of both the mobile and stationary phases. More advanced computer techniques allow combined optimizations of the mobile phase, pH and the content of the organic modifying reagent in HPLC analyses [18].

For optimization of the mobile phase for the HPLC of zinc(II) carboxylates the PRISMA model [19,20], utilizing combinations of the elution strength of the solvents (based on the Snyder scale [21]), in RP-HPLC of the substances examined was used. By modifying the composition of the three solvents and water, in relation to the capacity factor of the substance analyzed, the optimum mobile phase composition for zinc(II) formate, acetate, propionate, butyrate and isobutyrate salts with bound N-donor ligands was selected by the PRISMA model.

EXPERIMENTAL

Chemicals

All solvents were of analytical-reagent grade. Dioxane, methanol (MeOH), 2-propanol (PrOH) and ethanol (EtOH) were purchased from Lachema (Brno, Czech Republic) and acetonitrile (ACN) from VEB Laborchemie (Apolda, Germany).

Redistilled water [prepared with an Ilmator system (Biplex, Jena, Germany)] had a conductivity of 0.4 μS.

All zinc(II) carboxylates tested were provided by the Department of Inorganic Chemistry, P.J. Safarik University (Košice, Slovak Republic).

Caffeine (1,3,7-trimethylxanthine), thiourea, phenazone (2,3-dimethyl-1-phenyl-3-pyrazolin-5-one) and nicotinic acid (3-pyridinecarboxylic acid) were purchased from Medika (Bratislava, Slovak Republic).

Instruments

An LCP 4000 high-pressure HPLC pump (ECOM, Prague, Czech Republic), a Model 2062.2 spectrophotometric detector (ECOM) and a GP 3 gradient programmer with a Model 7125 injection valve with a 20-μl sample loop (Rheodyne, Berkeley, CA, USA) were used.

Analyses were carried out on the following columns: (A) LiChrosorb RP-8 (stainless steel, 250 × 4.6 mm I.D., $d_p = 7$ μm) and (B) LiChrosorb RP-18 (stainless steel, 250 × 4.6 mm I.D., $d_p = 7.5$ μm), both purchased from Knauer (Berlin, Germany). Results of the analyses were evaluated by means of an APEX on-line computer integrator and the program SOLVENT (ECOM).

Preparation of stock solutions

Solutions of the analyte substances (Table I) were prepared by weighing 5 mg of each compound into a 10-ml volumetric flask and dissolving in and diluting to volume with redistilled water. Mixtures 1 (pure ligands), 2 (formates), 3 (acetates), 4 (propionates), 5 (butyrates) and 6 (isobutyrates) were prepared by dissolving 5 mg of each compound in and diluting to volume with redistilled water in a 10-ml glass volumetric flask.

Conditions of analysis

All HPLC analyses were run under the following conditions: flow-rate of mobile phase, 1 ml/min; detection wavelength, 253 nm; detector temperature, 25°C; sample amount injected, 20 μl; and elution mode, isocratic.

Analysis

A detailed description of the procedure by which a suitable composition of the mobile phase is selected by means of the PRISMA model can be found in the literature [19,20]. The method can be summarized as follows:

(1) Selection of the three solvents suitable for analysis according to the Snyder classification.

(2) The solvent strength is first adjusted at selectivity point $P_S = 333$. At the determined solvent strength (S_{T1}), the k' values of the selectivity points representing four solvent combinations along the edges of the triangle between the basic selectivity points (433–343–334) are measured.

(3) From each of the four measured selectivity points along a line, the mathematical function $k' = aP_s^2 + bP_s + c$ for each zinc(II) carboxylate was calculated.

(4) The strategy in points (1) and (3) is repeated at two other solvent strength levels. The difference between the three solvent strength levels should not exceed 10–15% [19,20].

(5) The optimum mobile phase composition was calculated by the SOLVENT program, which optimizes mobile phase composition on the principle of finding the isoeluotropic plane in the tetrahedron of the solvents in the corners of the triangle. It is the plane on which the composition of the mobile phase gives a similar solvent and elution strength. The position of the plain varies according to the nature of the mixtures that are to be separated.

To find the optimum means finding a plane inside the PRISMA on which the peaks of the single substances have a capacity factor in the range 1–10 and chromatographic resolution is suitable.

The program SOLVENT allows the optimum mobile phase composition to be found after seven chromatographic analyses.

RESULTS AND DISCUSSION

From the results of preliminary TLC analyses, it follows that formates start to separate in mobile phases containing acetonitrile ($S_T = 5.8$), methanol ($S_T = 5.1$), dioxane ($S_T = 4.8$) and water ($S_T = 10.2$). For acetate separations, a mixture of acetonitrile, methanol, 2-propanol ($S_T = 3.9$) and water appears to be suitable. Acceptable propionate, butyrate and isobutyrate separation takes place in methanol, dioxane and water mixtures as mobile phases. On the basis of these results, HPLC analysis of the zinc(II) carboxylates with N-donor ligands was started on the RP-8 and RP-18 columns. In the next step, gradient elution with ACN–water, MeOH–water, dioxane–water and PrOH–water was tested to predict the isocratic composition of the mobile phase. It can then be stated which plane inside the PRISMA can be used for optimization of the mobile phase composition. On a plane with $S_T = 0.5$ or lower, the capacity factors of the individual compounds tested were higher than 10. Increasing the S_T values to 1.95 and 2.4 led to more effective results. Subsequently, in zinc(II) carboxylate analyses on the LiChrosorb RP-8 and RP-18 columns the following mobile phases were used: (A) ACN–MeOH–dioxane–water ($S_T = 2.4$, $P_S = 333$, 181, 118, 811, 334, 343, 433, 136, 163, 316, 361, 613, 631) and (B) ACN–MeOH–PrOH–water [$S_T = 1.95$, P_S as for (A)].

Zinc(II) carboxylate analyses on the RP-8 column with mobile phase A revealed insufficient separation of the individual compounds and co-elution of nicotinic acid and thiourea salts. Using mobile phase B (on the same column), the

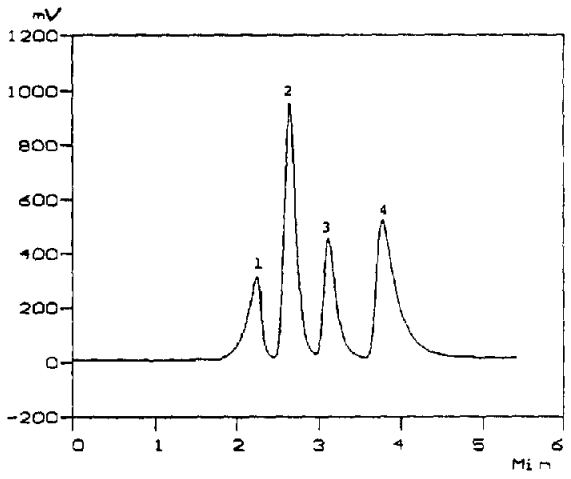

Fig. 1. HPLC of ligand mixture obtained on LiChrosorb RP-18 with mobile phase ACN–MeOH–PrOH–water (P_S = 343, S_T = 1.95), detection at 253 nm. Peaks: 1 = nicotinic acid; 2 = thiourea; 3 = caffeine; 4 = phenazone.

chromatographic resolution of the individual compounds in the mixtures was low with increasing capacity factors. In this system, partial separation of the mixture of compounds 1 and 10 [ligand and its zinc(II) carboxylate salt] was also observed.

Analyses on the RP-18 column with mobile phase A showed insufficient separation of thiourea- and caffeine-bound zinc(II) carboxylates, and the thiourea carboxylate peaks were non-symmetric.

Dioxane-containing mobile phases (the same selectivity points and elution strength) are not suitable for HPLC analysis of zinc(II) carboxylate mixtures. Probably decomposition of the original substances occurs or substitution of dioxane into the coordination sphere of the zinc(II) carboxylate salts takes place.

Mobile phase B appears to be more suitable

TABLE I

COMPOUNDS TESTED AND RETENTION TIMES (t_R) AND CAPACITY FACTORS (k') OF INDIVIDUAL COMPOUNDS ANALYSED ON LICHROSORB RP-18

Mobile phase: P_S = 333, ACN–MeOH–PrOH–water (10.09:11.47:15:63.44, v/v), S_T = 1.95.

No.	Compound	t_R (min)	k'
1	Caffeine (caff)	3.17	1.02
2	Thiourea (tu)	2.61	0.66
3	Phenazone (phen)	3.85	1.45
4	Nicotinic acid (nica)	2.21	0.41
5	$Zn(HCOO)_2(tu)$	2.55	0.62
6	$Zn(HCOO)_2(tu)_2(H_2O)_x$	2.55	0.62
7	$Zn(HCOO)_2(nica)(H_2O)_x$	2.23	0.42
8	$Zn(HCOO)_2(nica)_2(H_2O)_x$	2.49	0.59
9	$Zn(HCOO)_2(caff)(H_2O)_{0.5}$	3.16	1.01
10	$Zn(HCOO)_2(caff)_2(H_2O)$	3.19	1.03
11	$Zn(CH_3COO)_2(tu)$	2.59	0.65
12	$Zn(CH_3COO)_2(tu)_2(H_2O)_x$	2.59	0.65
13	$Zn(CH_3COO)_2(phen)_2(H_2O)_{1.5}$	4.00	1.55
14	$Zn(CH_3COO)_2(nica)_2(H_2O)_x$	2.56	0.63
15	$Zn(CH_3COO)_2(caff)(H_2O)_{2.5}$	3.28	1.09
16	$Zn(CH_3COO)_2(caff)_2(H_2O)_{3.5}$	3.35	1.13
17	$Zn(CH_3CH_2COO)_2(tu)_2$	2.68	0.71
18	$Zn(CH_3CH_2COO)_2(caff)_2$	3.56	1.27
19	$Zn[CH_3(CH_2)_2COO]_2(tu)$	2.69	0.71
20	$Zn[CH_3(CH_2)_2COO]_2(tu)_2$	2.89	0.84
21	$Zn[CH_3(CH_2)_2COO]_2(caff)_2$	3.68	1.34
22	$Zn[(CH_3)_2(CH_2)COO]_2(tu)$	2.73	0.74
23	$Zn[(CH_3)_2(CH_2)COO]_2(tu)_2$	2.94	0.87
24	$Zn[(CH_3)_2(CH_2)COO]_2(caff)$	3.29	1.10
25	$Zn[(CH_3)_2(CH_2)COO]_2(caff)_2$	3.60	1.29

for zinc carboxylate separations on the RP-18 column. All compounds chromatographed eluted within 5 min with acceptable chromatographic resolution (Fig. 1). At the selectivity point 118 splitting of the thiourea peak occurred and this effect was also observed with thiourea zinc(II) carboxylates.

The most advantegeous composition of the mobile phase for the separation of the compounds under examination is the composition found at the selectivity point $P_S = 334$ or $P_S = 343$.

The retention parameters (capacity factors of first and last peaks, volume of solvent, retention time of first and last peaks) obtained in zinc(II) carboxylate analyses (Table I) were used as input data for the SOLVENT program. By regression analysis a quadratic function was found that describes how the capacity factor k' depends on mobile phase composition (given by the P_S value) at constant S_T. From Fig. 2 it follows that an increasing content of ACN in the mobile phase causes anomalities in the retention behaviour of pure caffeine (as a ligand), zinc(II) formate with caffeine and also zinc(II) acetate with caffeine (the capacity factor and analysis time increase). The retention behaviour of zinc(II) propionate, butyrate and isobutyrate (with bound caffeine) is more similar to that of the compounds with other ligands. Increasing the ACN content and a parallel PrOH decrease (Fig. 3) result in higher capacity factors for phenazone and its zinc(II) acetate salt. The k' values for all salts with nicotinic acid show a slight decrease. The same anomaly was observed in the retention behaviour of zinc(II) caffeine salts. Analysis of caffeine and phenazone is also more time consuming in the mobile phase at selectivity point 181. The optimum mobile phase compositions for individual zinc(II) carboxylate HPLC analyses are given in Table II.

The hydrophobicity of the compounds examined increases with the increase in the aliphatic chain length of the anion, being evident

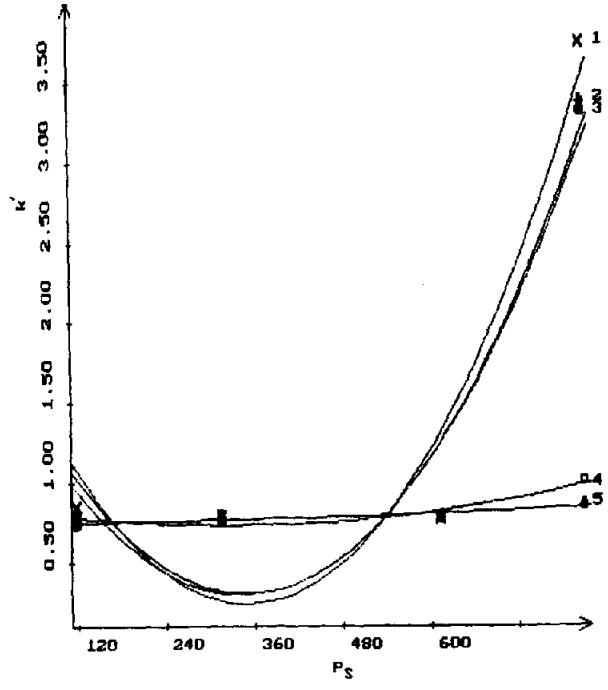

Fig. 2. Effect of P_S changes on the k' of the zinc(II) carboxylates. 1 = Pure caffeine; 2 = $Zn(HCOO)_2(caff)_2$; 3 = $Zn(CH_3COO)_2(caff)_2$; 4 = $Zn[CH_3(CH_2)_2COO]_2(caff)_2$; 5 = $Zn[(CH_3)_2CH_2COO]_2(caff)_2$.

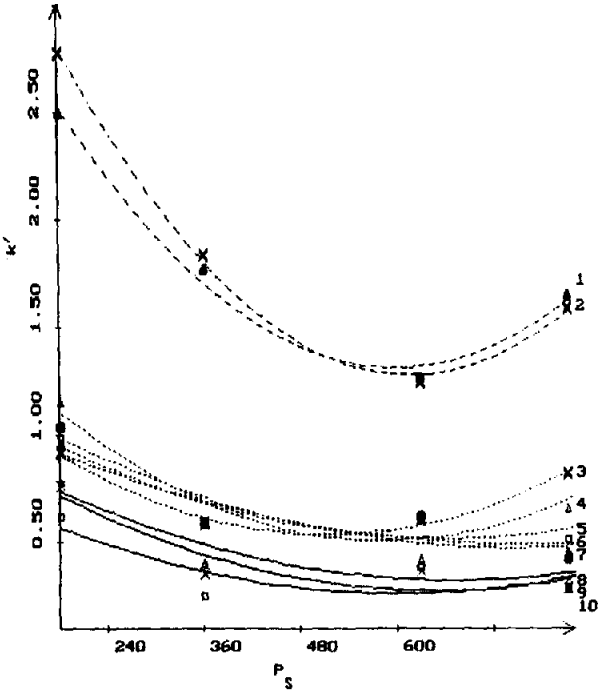

Fig. 3. Effect of mobile phase composition on the k' of zinc(II) carboxylates (P_S = 118, 163, 181, 316, 361, 613, 631, 811). 1 = Phenazone; 2 = $Zn(CH_3COO)_2(phen)_2$; 3 = thiourea; 4 = $Zn[CH_3(CH_2)_2COO]_2(tu)_2$; 5 = $Zn[(CH_3)_2CH_2COO]_2(tu)_2$; 6 = $Zn(HCOO)_2(tu)_2$; 7 = $Zn(CH_3COO)_2(tu)_2$; 8 = nicotinic acid; 9 = $Zn(CH_3COO)_2(nica)_2$; 10 = $Zn(HCOO)_2(nica)_2$.

TABLE II

OPTIMUM MOBILE PHASE COMPOSITION FOR SEPARATION OF ZINC(II) CARBOXYLATES ON RP-18 COLUMN, $S_T = 1.95$

Compound[a]	Mobile phase composition	P_S
4, 8, 14, 7	ACN–MeOH–PrOH–H_2O (26.9:3.83:5.0:64.27, v/v)	811
2, 5, 6, 11, 12, 17 19, 20, 22, 23	ACN–MeOH–PrOH–H_2O (20.2:3.8:15.0:61.0, v/v)	613
1, 9, 10, 15, 16, 18, 21, 24, 25	ACN–MeOH–PrOH–H_2O (20.2:3.8:15.0:61.0, v/v)	613
3, 13	ACN–MeOH–PrOH–H_2O (3.4:3.8:40.0:52.8, v/v)	118

[a] See Table I.

already in the analysis of propionates, in contrast to the formates and acetates, whose hydrophobicity is the same as for the pure ligand (Fig. 4).

The problem of the good chromatographic resolution of zinc(II) carboxylates (with an N-donor ligands) and its pure ligand or zinc(II) carboxylate–zinc(II) carboxylate mixtures (differing in their ligand numbers) is reminiscent of the problem in positional isomer separations [22]. For the identification and isolation of co-eluting components, the deconvolution process, based on the principal component analysis (PCA) technique [23], is frequently used. If the fact is taken into consideration that in mixtures consisting of a dissolved pure ligand and a corresponding zinc(II) carboxylate salt, a change in its ligand numbers or mutual ligand exchange may occur in the aqueous solution (complexation equilibria), the analysis of mixtures containing a zinc(II) carboxylate salt and the same ligand presents a problem which must be solved before starting tests on biological activity.

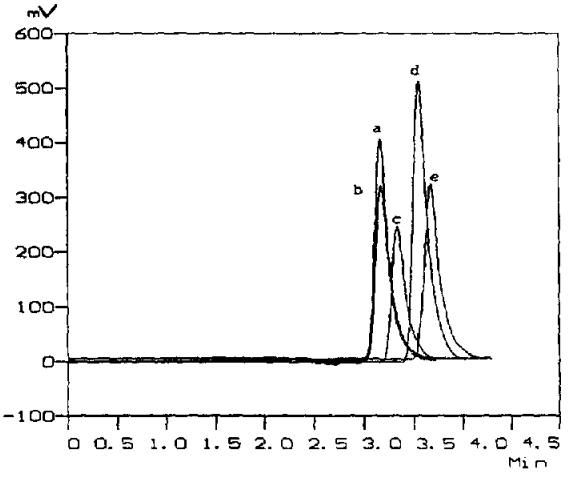

Fig. 4. Overlay of caffeine zinc(II) carboxylates showing aliphatic chain effect on the separation of individual compounds. Peaks: a = caffeine; b = zinc(II) formate with caffeine; c = zinc(II) propionate with caffeine; d = zinc(II) buthyrate with caffeine; e = zinc(II) isobutyrate with caffeine.

The mobile phase composition for the separation of zinc(II) carboxylates containing the same ligand in the molecule but with a different ligand number from a mixture (which mutually co-elute) has not yet been elucidated.

CONCLUSIONS

Novel $Zn(RCOO)_2 \cdot L_n \cdot q(H_2O)$ compounds were analysed on RP-8 and RP-18 columns. The optimum mobile phase composition for HPLC analysis of novel $Zn(RCOO)_2 \cdot L_n \cdot q(H_2O)$ compounds was chosen by using the PRISMA mathematical model and the program SOLVENT.

Good separations of the compounds observed were obtained with mobile phases containing acetonitrile, methanol, 2-propanol and water, at different P_S and constant S_T. Dioxane-containing mobile phases caused co-elution of zinc(II) carboxylates with nicotinic acid and thiourea. Separation on the RP-18 column is more effective than on the RP-8 column.

Some deviations in the retention behaviour of zinc(II) caffeine carboxylates were found and

analyses of caffeine and phenazone are more time consuming in mobile phase with $P_S = 181$. Gradient elution is also time consuming and does not give the required results.

A high 2-propanol content present in the mobile phase causes splitting of the thiourea and its zinc(II) carboxylate peaks.

The optimum mobile phase composition lies near to the acetonitrile corner of the solvent triangle and the area of optimal P_S is specified by the selectivity points 811, 613, 433, 344 and 631.

The results obtained (together with mobile phase flow optimization and confirmation of identity of each analyte separated) provide some of the information required before it will be possible to start analyses of zinc(II) carboxylates in the biological samples.

ACKNOWLEDGEMENT

This work is supported by the Slovak Ministry of Education Grant No. 1/990917/93 and it is gratefully acknowledged. The authors wish to thank Mr. Formanek from ECOM for the devices he provided. The authors also thank Miss Marta Stročeková for technical assistance.

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