

## Note

# Studies on the separation of Al<sup>III</sup>, Co<sup>III</sup>, Cr<sup>III</sup> and Fe<sup>III</sup> complexes with acetylacetonone by reversed-phase high-performance liquid chromatography

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The separation and determination of metal ions in the form of chelates of fluorinated analogues of acetylacetonone (HAA) by gas chromatography (GC) are well known<sup>1–4</sup>, but the application of reversed-phase high-performance liquid chromatography (RP-HPLC) to the separation and determination of simple HAA metal chelates has not yet been successfully demonstrated<sup>5</sup>.

The separation of  $\beta$ -diketone metal chelates, including metal acetylacetonates [M(AA)<sub>n</sub>], by HPLC was first reported by Huber and Kraak<sup>6</sup>. They employed liquid–liquid partition chromatography involving a ternary solvent system consisting of water, methanol and 2,2,4-trimethylpentane. They established the conditions necessary to separate four to six metal chelates of Be<sup>II</sup>, Al<sup>III</sup>, Cr<sup>III</sup>, Fe<sup>III</sup>, Co<sup>III</sup>, Ni<sup>II</sup>, Cu<sup>II</sup>, Zn<sup>II</sup>, Co<sup>II</sup>, Zr<sup>IV</sup> and Ru<sup>III</sup>. The first attempt to separate  $\beta$ -diketone chelates by RP-HPLC was reported by Tollinche and Risby<sup>7</sup>. Although their studies demonstrated a successful separation of the chelates by normal-phase HPLC, they were completely unsuccessful in separating the complexes by RP-HPLC.

RP-HPLC was successfully used by Gurira and Caar<sup>8</sup> for the separation of Mn<sup>II</sup>, Be<sup>II</sup>, Co<sup>III</sup>, Cr<sup>III</sup>, Rh<sup>III</sup>, Ir<sup>III</sup>, Pd<sup>II</sup> and Pt<sup>II</sup> acetylacetonates with Supelco C<sub>18</sub> and Ultrasphere C<sub>18</sub> columns. They obtained good separations of these chelates (4–7 components) using methanol–water or acetonitrile–water mixtures as mobile phases.

This paper reports the separation of mixtures of acetylacetonates of Co<sup>III</sup>, Cr<sup>III</sup>, Al<sup>III</sup> and Fe<sup>III</sup> by RP-HPLC with columns containing LiChrosorb packings modified with RP-2, RP-8 and RP-18 hydrocarbon bonded phases and methanol–water or acetonitrile–water mobile phases.

## EXPERIMENTAL

A Liquochrom Model 2010 high-performance liquid chromatograph (Labor, Budapest, Hungary), equipped with a UV detector (180–440 nm), was used. Eight steel columns (25 or 10 cm × 4.6 mm I.D.) were used with LiChrosorb packings (10, 7 or 5  $\mu$ m) modified with RP-2, RP-8 or RP-18 hydrocarbon bonded phases (ZOCh,

TABLE I  
THE CHARACTERISTICS OF COLUMNS FOR SEPARATION OF  $M(AA)_3$

Column No.	Bonded stationary phase	Column length (cm)	Particle size ( $\mu\text{m}$ )	High equivalent to a theoretical plate (mm)	Flow-rate (linear) of mobile phase (cm/s)	
					$\text{CH}_3\text{OH}-\text{H}_2\text{O}$ (40:60)	$\text{CH}_3\text{CN}-\text{H}_2\text{O}$ (30:70)
1	RP-18	25	10	0.254	0.250	0.228
2	RP-8	25	10	0.285	0.209	0.188
3	RP-2	25	10	0.283	0.245	0.208
4	RP-2	25	7	0.266	0.233	0.227
5	RP-2	25	5	0.290	0.157	0.190
6	RP-18	10	10	0.244	0.247	0.251
7	RP-18	10	7	0.230	0.254	0.220
8	RP-18	10	5	0.247	0.166	0.175

Lublin, Poland). Table I gives the characteristics of the columns. UV spectra of the metal chelates were obtained on a Specord UV-VIS instrument (Carl Zeiss, Jena, G.D.R.).

The reagents used were pure chromium acetylacetonate,  $\text{Cr}(\text{C}_5\text{H}_7\text{O}_2)_3$  (Serva, Heidelberg, F.R.G.), cobalt acetylacetonate for synthesis,  $\text{Co}(\text{C}_5\text{H}_7\text{O}_2)_3$  (Merck-Schuchard, Darmstadt, F.R.G.), aluminium acetylacetonate,  $\text{Al}(\text{C}_5\text{H}_7\text{O}_2)_3$ , and iron(III) acetylacetonate,  $\text{Fe}(\text{C}_5\text{H}_7\text{O}_2)_3$ , both prepared by us by the method described by Charles and Pawlikowski<sup>9</sup>, spectrally pure methanol (POCh, Gliwice, Poland) and acetonitrile for chromatography (Merck-Schuchard). The mobile phases were methanol-water (50:50 and 40:60, v/v) and acetonitrile-water (50:50, 40:60, 30:70 and 25:75, v/v). The solvents were thoroughly mixed with redistilled water and degassed on an ultrasonic bath under reduced pressure for 10–15 min.

Solutions of  $M(AA)_3$  were prepared by dissolving suitable amounts of chelates in methanol or acetonitrile and diluting to concentrations of  $10^{-4}$ – $10^{-5}$  M. UV absorption bands ( $\lambda_{\text{max}}$ ) of the investigated solutions of  $\text{Co}(AA)_3$ ,  $\text{Cr}(AA)_3$ ,  $\text{Al}(AA)_3$  and  $\text{Fe}(AA)_3$  were 256, 332, 294 and 276 nm, respectively, in acetonitrile solutions and 252, 326, 282 and 270 nm, respectively, in methanol solutions. Sample solutions of the complexes were injected onto the column (20  $\mu\text{l}$ ) and the absorbances of the mixture complexes was detected at 254, 280 and 310 nm, but the best results in peak height were obtained at 280 nm.

## RESULTS AND DISCUSSION

Measurements of the retention times of  $\text{Co}(AA)_3$ ,  $\text{Cr}(AA)_3$ ,  $\text{Al}(AA)_3$  and  $\text{Fe}(AA)_3$  acetylacetonates were carried out on eight columns with methanol-water and acetonitrile-water mobile phases at flow-rates in the range 0.7–2.0  $\text{cm}^3/\text{min}$ . Depending on the mobile phase composition (see above), these flow-rates forced the slowest component,  $\text{Cr}(AA)_3$ , through the column in less than 15 min. Table II gives the measured retention times of  $M(AA)_3$  for acetonitrile-water (30:70) and methanol-water (40:60) as mobile phases. Under the conditions employed, the chelates gave

TABLE II

RETENTION TIMES OBTAINED FOR  $M(AA)_3$  IN ACETONITRILE-WATER (30:70) AND METHANOL-WATER (40:60)

Column No.	Flow-rate of mobile phase ( $cm^3/min$ )	Retention time (min)						
		Acetonitrile-water (30:70)			Methanol-water (40:60)			
		Co(AA) <sub>3</sub>	Al(AA) <sub>3</sub>	Cr(AA) <sub>3</sub>	Fe(AA) <sub>3</sub>	Al(AA) <sub>3</sub>	Co(AA) <sub>3</sub>	Cr(AA) <sub>3</sub>
1	1.5	7.75	10.67	13.00	—	—	11.17	15.00
2	1.5	7.50	10.50	12.17	—	—	11.08	14.62
3	1.5	6.58	8.92	10.42	3.50	4.58	10.00	13.00
4	1.5	5.13	6.70	7.72	3.67	4.17	9.82	12.58
5	1.5 (1.0) <sup>a</sup>	6.20	8.12	9.47	5.75	6.33	8.82	10.82
6	1.5	3.45	5.03	6.08	1.93	3.01	5.17	7.17
7	1.5	3.75	5.33	6.50	3.02	4.01	6.42	9.17
8	0.9	4.45	6.18	7.58	3.00	3.67	6.82	9.50

<sup>a</sup> Flow-rate of 1.0  $cm^3/min$  applies to the methanol-water (40:60) eluent.

sharp symmetric peaks, with the exception of  $Fe(AA)_3$ , which showed slight tailing with both mobile phases, and  $Al(AA)_3$ , which showed the same phenomenon with methanol-water. According to our results, consistently with those of Huber and Kraak<sup>6</sup>, these chelates tend to dissociate in aqueous solutions. The elution order of the  $Co(AA)_3$  and  $Al(AA)_3$  chelates is influenced by the type of mobile phase. In particular,  $Al(AA)_3$  elutes first and  $Co(AA)_3$  elutes next with methanol-water, but with acetonitrile-water this order is reversed.

Figs. 1 and 2 show chromatograms of  $M(AA)_3$  mixtures. The mixtures were well

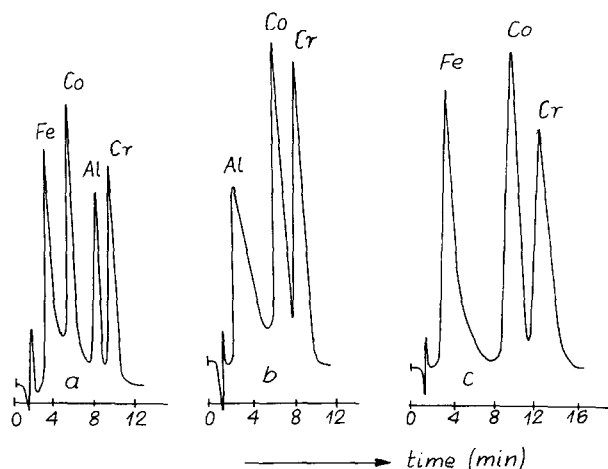


Fig. 1. Chromatograms of  $M(AA)_3$  mixtures obtained on columns containing LiChrosorb RP-2. Mobile phase: (a) acetonitrile-water (30:70); (b, c) methanol-water (40:60). Flow-rate: (a, c) 1.5  $cm^3/min$ ; (b) 2.0  $cm^3/min$ . (a)  $Fe(AA)_3 + Co(AA)_3 + Al(AA)_3 + Cr(AA)_3$  (3:1:4:2); 20- $\mu l$  sample volume contains  $4.21 \cdot 10^{-7}$  g Fe,  $2.38 \cdot 10^{-7}$  g Co,  $6.74 \cdot 10^{-7}$  g Al and  $4.2 \cdot 10^{-7}$  g Cr. (b)  $Al(AA)_3 + Co(AA)_3 + Cr(AA)_3$  (3:1:2);  $3.25 \cdot 10^{-7}$  g Al,  $2.37 \cdot 10^{-7}$  g Co and  $4.23 \cdot 10^{-7}$  g Cr. (c)  $Fe(AA)_3 + Co(AA)_3 + Cr(AA)_3$  (4:1:2);  $8.98 \cdot 10^{-7}$  g Fe,  $2.37 \cdot 10^{-7}$  g Co and  $4.23 \cdot 10^{-7}$  g Cr.

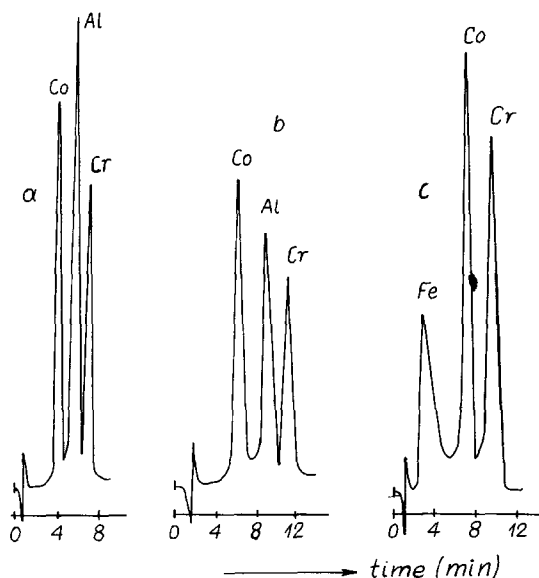


Fig. 2. Chromatograms of  $M(AA)_3$  mixtures obtained on columns containing LiChrosorb RP-18. Mobile phase: (a, b) acetonitrile-water (30:70); (c) methanol-water (40:60). Flow-rate: (a, c)  $2.0 \text{ cm}^3/\text{min}$ ; (b)  $1.5 \text{ cm}^3/\text{min}$ . (a, b)  $\text{Co}(AA)_3 + \text{Al}(AA)_3 + \text{Cr}(AA)_3$  (1:3:2);  $2.38 \cdot 10^{-7} \text{ g Co}$ ,  $3.16 \cdot 10^{-7} \text{ g Al}$  and  $4.2 \cdot 10^{-7} \text{ g Cr}$ . (c)  $\text{Fe}(AA)_3 + \text{Co}(AA)_3 + \text{Cr}(AA)_3$  as in Fig. 1a.

separated with the columns and mobile phases employed. A mixture of  $\text{Co}(AA)_3$ ,  $\text{Cr}(AA)_3$ ,  $\text{Al}(AA)_3$  and  $\text{Fe}(AA)_3$  was separated on column 3 modified with RP-2 using acetonitrile-water (30:70) (Fig. 1). A mixture of  $\text{Co}(AA)_3$ ,  $\text{Al}(AA)_3$  and  $\text{Cr}(AA)_3$  was successfully separated on all columns using the same mobile phase. Methanol-water (40:60) was used for the separation of another three-component chelate mixture, but the separation of  $\text{Co}(AA)_3$  and  $\text{Al}(AA)_3$  was not complete.

TABLE III

COMPARISON OF CAPACITY FACTORS ( $k'$ ) AND RELATIVE RETENTIONS ( $\alpha$ ) FOR  $M(AA)_3$

Column No.	Column packing	Mobile phase	$k'$			$\alpha$		
			$\text{Co}(AA)_3$	$\text{Al}(AA)_3$	$\text{Cr}(AA)_3$	Al/Co	Cr/Al	Cr/Co
1	RP-18	$\text{CH}_3\text{CN}-\text{H}_2\text{O}$ (30:70)	3.15	4.82	5.96	1.53	1.24	1.89
2	RP-8		2.40	3.72	4.50	1.55	1.21	1.87
3	RP-2		2.28	3.45	4.19	1.51	1.21	1.84
4	RP-2		1.80	2.65	3.20	1.47	1.21	1.78
5	RP-2		2.27	3.45	4.23	1.52	1.23	1.86
6	RP-18		4.20	6.55	8.15	1.56	1.24	1.94
7	RP-18		3.95	6.10	7.65	1.54	1.25	1.94
8	RP-18		3.70	5.53	6.95	1.50	1.25	1.94
1	RP-18	$\text{CH}_3\text{CN}-\text{H}_2\text{O}$ (25:75)	4.68	7.50	9.43	1.60	1.26	2.01
6	RP-18		4.85	7.85	9.85	1.62	1.25	2.03
7	RP-18		5.75	9.60	12.05	1.67	1.25	2.09
8	RP-18		4.10	6.45	8.16	1.57	1.26	1.99

Table III gives the capacity factors ( $k'$ ) and relative retentions ( $\alpha$ ) calculated according to our results, consistent with Hamilton and Sewell<sup>10</sup>. These parameters were calculated for all columns and all mobile phases, but Table III just gives the data for acetonitrile–water (30:70) for all columns and for acetonitrile–water (25:75) for columns packed with LiChrosorb RP-18. The  $k'$  values for column 1 range from 1.4 to 2.2 for acetonitrile–water (50:50) and increase to 4.7–9.4 for acetonitrile–water (25:75). All the  $k'$  values for the other columns are limited to the range 1.8–8.15. The  $k'$  values are higher for the 10-cm columns (columns 6–8) using acetonitrile–water (25:75).

The relative retention ( $\alpha$ ) varies slightly, from 1.4 to 2.0 in acetonitrile–water and from 1.25 to 1.40 in methanol–water. The lowest  $\alpha$  values of 1.21–1.25 were found for Cr(AA)<sub>3</sub> and for Al(AA)<sub>3</sub>. The  $\alpha$  values for Al(AA)<sub>3</sub> range from 1.78 to 1.94. Lower  $\alpha$  values (1.29–1.43) were obtained with methanol–water (40:60) for Cr(AA)<sub>3</sub> and Co(AA)<sub>3</sub> chelates.

The separation of M(AA)<sub>3</sub> was performed using different particle sizes of the support (columns 4–8) and a 10-cm column (columns 6–8). The retention times and capacity factors found for columns of the same length and particle size (7 or 10  $\mu\text{m}$ ) indicate similar separation effects. Retention times obtained on the same support (columns 1 and 6) depends on the column length. In particular, the retention times of M(AA)<sub>3</sub> on a 25-cm column are 2.15 times longer than those on a 10-cm column. The separation effects on these shorter columns were consistent with those on the others tested with regard to the chromatograms,  $k'$  values and relative retentions.

## CONCLUSIONS

Optimum parameters for the separation of M(AA)<sub>3</sub> mixtures were established. The resolution time, depending on the column type and flow-rate (1.5–2.0  $\text{cm}^3/\text{min}$ ), was 6–10 min. The effectiveness of the separation is independent of the column length in the range 10–25 cm, but the column length influences the retention time (proportional relationship).

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