

Comparative analysis of organic acids located on the surface of natural building stones by high-performance liquid, gas and ion chromatography

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(First received December 15th, 1989; revised manuscript received April 6th, 1990)

ABSTRACT

Highly polar carboxylic acids located on stone surfaces were analysed with regard to their potential impact on the deterioration of historical sites and monuments. A method for the elution of several organic acids from natural building stones is described. The performance of this method was confirmed by comparative high-performance liquid, gas and ion chromatographic analyses of the extracts obtained from prepared stone samples.

INTRODUCTION

Historical sites and monuments constructed from natural building stones deteriorate by several natural and anthropogenic mechanisms of weathering^{1,2}. Buildings constructed from sandstone or marble are especially prone to decay.

Microorganisms such as fungi, bacteria and algae grow on weathered surfaces of buildings made of natural stones; macroorganisms such as moss, lichens and higher plants are also often observed³. Several strains of aerobic microorganisms produce mineral acids; in addition, complexing organic acids can be secreted as metabolites of the citric acid cycle, anaerobic glycolysis and other energetic cycles⁴. These metabolites may cause destruction by dissolution of minerals, modification of mineral structures and extension of pore sizes^{5,6}.

For quantitative determination, carboxylic acids had to be extracted from previously pulverized stones by a suitable method. The samples were ground in a mortar and pestle to avoid decomposition and losses of carboxylic acids, which occur as a consequence of thermal strain if globe mills or mortar mills are used.

The high calcium content of some building stones (up to 30%) was a major problem with regard to the extraction process, because salts formed by the expected carboxylic acids with calcium ions are of very low solubility. Previously described extraction methods^{7–10} did not prove suitable for the extraordinary matrix.

We have shown that mild extraction conditions are necessary to avoid decomposition²². The best results were obtained by extraction of acids using cation exchangers. As several commercially available polymeric cation exchangers of analytical-reagent grade display strong chromatographic interferences even if extensively purified, we turned to inorganic ion exchangers. Zeolite A (Na⁺ form) had the highest specificity for calcium and other bivalent cations and turned out to be sufficiently purifiable by common laboratory methods.

Methods for determining carboxylic acids by high-performance liquid chromatography (HPLC) have been frequently described. Many workers have tried to enhance the ultraviolet sensitivity by derivatization^{11,12}. No satisfactory results were obtained for highly polar carboxylic acids, especially at low concentrations.

Separation of the compounds was first tried by using ion-pairing reagents¹³ on reversed phases. High resolution but poor detection limits were characteristics of this method. Direct separation on reversed phases^{14,15} is not sufficiently specific for anions in a complex matrix. A combined single-column ion chromatographic-ion exclusion chromatographic method¹⁶ could be adapted to the complex matrix of our samples and thereby solve this special separation problem. Detection limits of 2–570 nmol/g were realized and a highly sufficient group separation was obtained.

Analysis of organic anions by ion-exchange chromatographic (IC) methods is subject to interference by inorganic anions and the strong retention of, *e.g.*, citrate. As an isocratic method can hardly deal with these difficulties, a suitable gradient system was developed. Shintani and Dasgupta¹⁷ described a sodium hydroxide gradient technique which could be adapted to the problem; increased solvent contamination was counteracted by on-line purification of the eluent with an anion-trap column.

Gas chromatographic (GC) analysis of carboxylic acids requires derivatization prior to analysis to increase the volatility of these polar compounds. In our case, the derivatization procedure had to deal with samples containing high concentrations of inorganic salts and at least trace amounts of water. As some of the acids in question are susceptible to decarboxylation, extensive heating had to be avoided.

Promising results were obtained with an esterification procedure employing *n*-butanol and concentrated sulphuric acid as reagents¹⁸; it is described as being especially suitable for samples containing inorganic salts and small amounts of water. As this method showed poor blanks, especially with regard to oxalic acid, acetyl chloride was used as a reagent instead of sulphuric acid^{9,19}. The results obtained after this variation were favourable, and the method established shows good limits of determination and easy handling, is not prone to disturbances and allows large numbers of samples to be processed.

EXPERIMENTAL

Standard solutions

Aqueous standard solutions of nine carboxylic acids were prepared to evaluate the efficiency of the extraction procedure. Oxalic, succinic and formic acid (E. Merck, Darmstadt, F.R.G.) and citric and tartaric acid (Riedel-de Haën, Seelze, F.R.G.) were of analytical-reagent grade. Propionic, fumaric and glyoxylic acid (E. Merck) were synthetic grade and malic acid (Riedel-de Haën) was labelled "biosynth". Water doubly distilled in an all-quartz apparatus. Concentrations of the three standard solutions (CA 1–3) are listed in Table I.

TABLE I
CARBOXYLIC ACID CONCENTRATIONS IN STANDARD SOLUTIONS

Acid	Concentration (mg/l)		
	CA 1	CA 2	CA 3
Formic	907	1896	3238
Propionic	1993	3987	9537
Citric	396	985	4138
Succinic	215	1995	3984
Oxalic	231	996	9847
Malic	993	1972	3943
Tartaric	991	1982	3968
Fumaric	26	401	2005
Glyoxylic	391	1140	3184

Stone samples

Three types of building stone often used at historical sites were selected with regard to their different contents of calcium carbonate: the limestone Krenzheimer Muschelkalk (K), the quartzite Worzeldorfer Quarzit (W) and the sandstone Ebenheidener Sandstein (E). The respective contents of the major bivalent cations as measured by inductively coupled plasma atomic emission spectrometry (ICP-AES) are given in Table II. Freshly quarried stones (Pressbau, Oberhausen, F.R.G.) were sawn into 16 ashlar of each type with weights varying from 3.13 to 6.75 g.

Four ashlar of each type were spiked with each of the CA 1–3 solutions. Four samples of each type of stone were chosen to determine blank values and were treated similarly to the spiked samples in the following process.

Extraction procedure

Zeolite A was purified prior to use as follows: 100 g of zeolite A (analytical-reagent grade; Fluka, Buchs, Switzerland) were shaken for 2 h with 500 ml of doubly distilled water and filtered by suction. This process was repeated until no relevant

TABLE II
CONTENTS OF BIVALENT CATIONS IN NATURAL BUILDING STONES DETERMINED BY ICP-AES

Cation	Concentration (g/kg)		
	Worzeldorfer Quarzit	Ebenheidener Sandstein	Krenzheimer Muschelkalk
Ca ²⁺	0.49	0.76	357.00
Mg ²⁺	0.84	2.37	1.75
Fe ²⁺	4.22	8.79	0.85
Ba ²⁺	0.56	0.41	0.08
Sr ²⁺	0.06	0.05	1.24

TABLE III

MAXIMUM CONCENTRATIONS OF CARBOXYLIC ACIDS IN EXTRACTS FROM SPIKED STONE SAMPLES (100% RECOVERY ASSUMED)

Acid	Concentration (mg/l)		
	I (spiked with CA 1)	II (spiked with CA 2)	III (spiked with CA 3)
Formic	4.54	9.48	16.20
Propionic	9.97	19.94	47.70
Citric	1.98	4.93	26.10
Succinic	1.08	9.98	19.92
Oxalic	1.16	4.98	49.25
Malic	4.97	9.86	19.72
Tartaric	4.96	9.91	19.84
Fumaric	0.13	2.01	10.03
Glyoxylic	1.96	5.70	15.95

chromatographic interferences were found in the filtrate. The purified zeolite A was dried overnight at 100°C.

For W and E, 1.0 g of stone powder, 10 ml of doubly distilled water and 2.0 g of zeolite A were placed in a 50-ml screw-capped vial. For K, with regard to its high calcium content, 0.5 g of stone powder, 10 ml of water and 5.0 g of zeolite A were taken. These samples were shaken (7 times per second) for 4 h. After deposition for 16 h they were filtered through a 0.2- μ m cellulose membrane (Millipore, Bedford, MA, U.S.A.).

Table III gives the carboxylic acid concentrations in the solutions obtained, corresponding to 100% extraction efficiency.

Instrumental

High-performance liquid chromatography as single-column ion chromatography.

The following equipment was used: pump, ConstaMetric III; detector, Spectro-Monitor D; integrator, MP 3000 (all from LDC-Milton Roy, U.S.A.); precolumn, Chromguard anion-exchange column (50 \times 3 mm I.D.) (Chrompack, Middelburg, The Netherlands); separation column, ION-300 (ICT, CA, U.S.A.); and a column oven. The separation conditions were 5 mM sulphuric acid, flow-rate 0.4 ml/min, 40°C and detection at 210 nm.

Gas chromatography. A sample of 0.1–1.0 ml, depending on concentration, was evaporated *in vacuo* at 30°C on a rotary evaporator. A 1.0-ml volume of 1-butanol (analytical-reagent grade) and 0.5 ml of acetyl chloride (synthetic grade) (both from E. Merck) were added. After 1.5 h at 25°C, 7 ml of water were added and the solution was extracted three times with 0.3 ml of 2,2,4-trimethylpentane (analytical-reagent grade; E. Merck). A 10.0- μ l volume of an internal standard solution containing 25.0 μ g/ml of *n*-eicosane (Supelco, Bellefonte, PA, U.S.A.) was added to the combined organic extracts. Finally, the sample was evaporated *in vacuo* without heating to a final volume of 0.1 ml.

A Model FV 4160 gas chromatograph equipped with a Model EI 490 electrometer, Model 430 temperature programmer, Model G1/M3 split injector and

a flame ionization detector (all from Carlo Erba, Milan, Italy) was used. The integrator was a C-R3A (Shimadzu, Tokyo, Japan). Compressed air and hydrogen (quality 5.0; Linde, Unterschleissheim, F.R.G.) were used as detector gases and the latter also used as carrier gas at a pressure of 40 kPa. The samples were separated on a Superox II column (Alltech Germany, Unterhaching, F.R.G.) (30 m \times 0.25 mm I.D.) with a 0.25- μ m film thickness. The temperature programme was 80°C start, increased at 30°C/min to 140°C, 3°C/min to 185°C and 15°C/min to 260°C, and a 10-min hold at 260°C.

Ion chromatography. The Model 4000i ion chromatograph (Dionex, Sunnyvale, CA, U.S.A.) includes a gradient programmer, pump and conductivity detector; a C-R3A integrator (Shimadzu) was used. The regenerant was 50 mM sulphuric acid (analytical-reagent grade; E. Merck) at a flow-rate of 4 ml/min. Eluent 1 was 0.75 mM sodium hydroxide solution (analytical-reagent grade; Baker, Phillipsburg, NJ, U.S.A.) and eluent 2 was 200 mM sodium hydroxide solution, with the following gradient: 0 min, 100%; 4 min, 100%; 8 min, 90%; 14 min, 83%; 20 min, 72%; and 30 min, 45% eluent 1; the flow-rate was 1 ml/min. ATC, HPIC-AG5A (5 μ m), HPIC-AS5A (5 μ m) and AMMS columns (all from Dionex) were used.

RESULTS AND DISCUSSION

This work had two major objectives: first to examine the efficiency of the proposed extraction method and second to investigate the reliability of the three chromatographic methods for this kind of sample. The results for Worzeldorfer Quarzit are presented in Figs. 1 and 2; they were standardized by subtraction of zero

Recovery

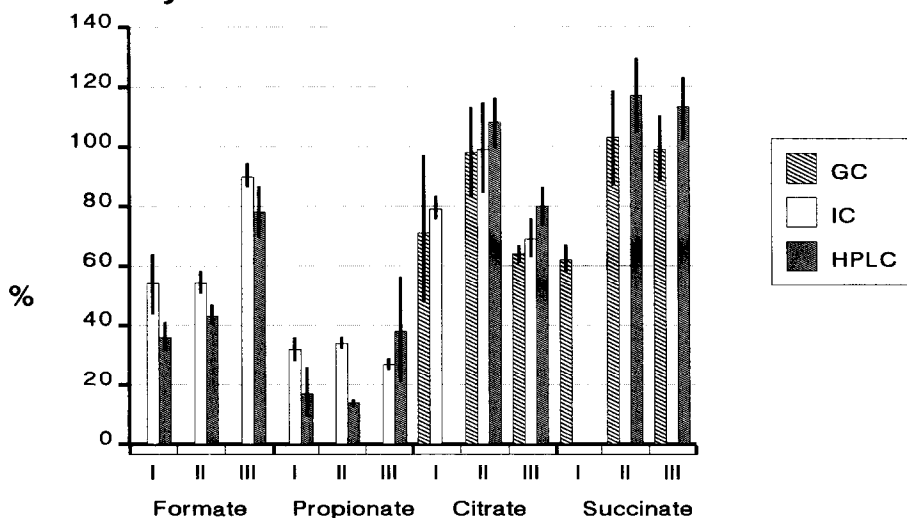


Fig. 1. Recoveries of formate, propionate, citrate and succinate from the quartzite Worzeldorfer Quarzit as determined by GC, IC and HPLC. The error bars represent relative standard deviations. I, II and III: sample concentrations are given in Table III.

Recovery

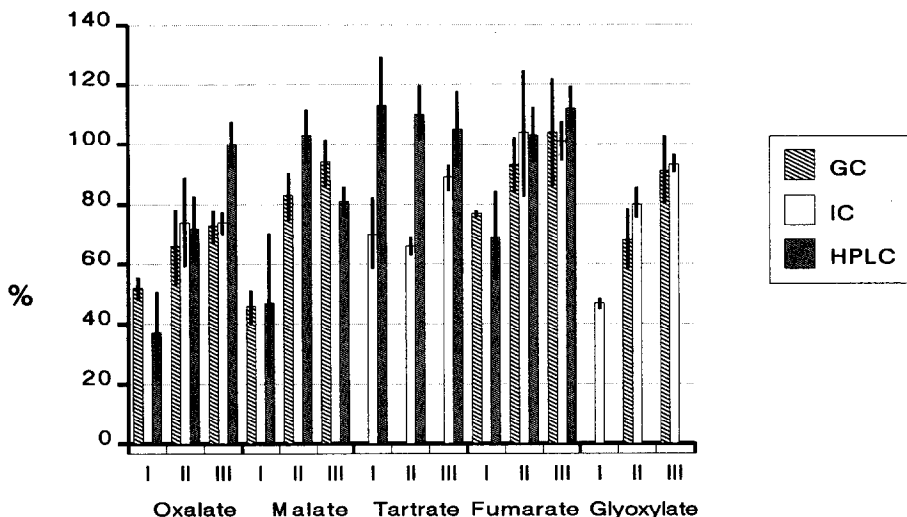


Fig. 2. Recoveries of oxalate, malate, tartrate, fumarate and glyoxylyate from the quartzite Worzeldorfer Quarzit as determined by GC, IC and HPLC. The error bars represent relative standard deviations. I, II and III: sample concentrations are given in Table III.

TABLE IV

RECOVERIES (*R*) AND RELATIVE STANDARD DEVIATIONS (R.S.D.) OF HIGHLY POLAR CARBOXYLIC ACIDS EXTRACTED FROM KRENZHEIMER MUSCHELKALK AND EBENHEIDENER SANDSTEIN

R.S.D. values are normalized by the recoveries.

Stone	Sample ^a	Method	Formic acid		Propionic acid		Citric acid	
			<i>R</i> (%)	R.S.D. (%)	<i>R</i> (%)	R.S.D. (%)	<i>R</i> (%)	R.S.D. (%)
Krenzheimer Muschelkalk	I	GC	nd ^b		nd		46	3
		IC	nd		nd		82	5
		HPLC	97	15	36	4	97	7
	II	GC	nd		nd		29	2
		IC	nd		84	18	111	14
		HPLC	110	21	79	7	104	24
	III	GC	nd		nd		48	18
		IC	nd		93	11	87	4
		HPLC	102	12	85	19	94	20
Ebenheidener Sandstein	I	GC	nd		nd		51	4
		IC	nd		43	7	73	9
		HPLC	101	7	39	7	nd	
	II	GC	nd		nd		84	12
		IC	97	14	25	4	nd	
		HPLC	63	6	13	4	110	23
	III	GC	nd		nd		67	16
		IC	105	5	24	2	81	5
		HPLC	73	7	23	1	82	8

^a Sample concentrations are given in Table III.

^b Not determined.

concentrations. The boxes show percentage recoveries and the error bars represent relative standard deviations (R.S.D.)²⁰, normalized by the rate of recovery:

$$\text{R.S.D. (\%)} = \frac{100}{\bar{x}} \sqrt{\frac{\sum(x_i - \bar{x})^2}{n - 1}}$$

The results for Krenzheimer Muschelkalk and Ebenheidener Sandstein are given in Table IV. Table V shows which chromatographic methods were applied to determine the different carboxylic acids.

Succinic, fumaric, tartaric and citric acid were successfully recovered even from K and from samples with the lowest concentrations of carboxylic acids; the recoveries range from about 70 to 100%. The different chromatographic methods give concordant results. Malic and especially oxalic acid display increasing recoveries from low to high concentration levels. This indicates that a small amount of calcium ions still exists in the extraction solutions containing zeolite A. However, the HPLC and GC data both show that about 75% of oxalic and malic acid were extracted when 50 $\mu\text{g/g}$ oxalic acid or 20 $\mu\text{g/g}$ malic acid were present in the stone sample.

At first glance, the low recoveries obtained for glyoxylic, propionic and formic acid are striking. They are obviously due to the drying process at 60°C, which led to partial vaporization of the volatile carboxylic acids. The degree of vaporization depends on the respective acidities of stones and acids; the alkaline limestone K shows no losses of formic acid and only slight losses of propionic acid. A small amount of

CARBOXYLIC ACIDS EXTRACTED FROM KRENZHEIMER MUSCHELKALK AND EBENHEIDENER

<i>Succinic acid</i>		<i>Oxalic acid</i>		<i>Malic acid</i>		<i>Tartaric acid</i>		<i>Fumaric acid</i>		<i>Glyoxylic acid</i>	
<i>R</i> (%)	<i>R.S.D.</i> (%)	<i>R</i> (%)	<i>R.S.D.</i> (%)	<i>R</i> (%)	<i>R.S.D.</i> (%)	<i>R</i> (%)	<i>R.S.D.</i> (%)	<i>R</i> (%)	<i>R.S.D.</i> (%)	<i>R</i> (%)	<i>R.S.D.</i> (%)
73	10	23	3	37	4	nd		77	1	29	7
nd		nd		nd		93	15	nd		109	9
nd		34	2	122	29	94	2	92	22	nd	
78	8	36	6	48	4	nd		91	14	37	8
nd		nd		nd		108	5	nd		nd	
102	3	74	4	60	27	67	8	109	7	nd	
100	16	66	11	98	10	nd		97	9	51	7
nd		nd		nd		115	7	nd		86	17
123	14	84	4	54	22	77	19	111	17	nd	
60	7	29	6	54	4	nd		nd		nd	
nd		nd		nd		87	16	nd		nd	
nd		30	2	60	10	108	35	77	1	nd	
95	7	61	7	86	3	nd		98	10	44	4
nd		74	3	nd		99	7	99	1	45	6
107	8	82	6	95	26	101	25	102	6	58	14
100	7	75	4	100	6	nd		97	14	63	13
nd		37	10	nd		99	11	102	11	59	6
113	9	92	9	27	6	94	18	105	7	nd	

formic acid but more than 50% of propionic acid were lost from the slightly acidic sandstone E. More than 60% of propionic acid and 30–60% of formic acid escaped from the more acidic quartzite W. Owing to the losses, no actual recovery data can be given for E and W. Nevertheless, a good extraction efficiency is indicated by data obtained from K, which is the most difficult matrix because of its very high calcium content; IC and HPLC gave corresponding results.

The thermal evaporation of volatile carboxylic acids indicates that vaporization of these compounds is also to be expected on buildings, especially on walls with a southern alignment where solar irradiation can cause great heat on the stone surface.

Glyoxylic acid is probably not vaporized, but decomposes during the drying process. This assumption was confirmed by previous storage experiments with spiked stone powder at -20°C , 6°C and at room temperature. For example, the starting concentration of glyoxylic acid decreased to well below 50% within 3 days at 23°C .

The suitability of the three chromatographic methods employed was judged by the following criteria: (a) interference with other compounds usually present in this matrix, (b) reproducibility of the results and (c) limits of determination. Figs. 3–5 show typical chromatograms of carboxylic acids extracted from stone samples by means of an aqueous suspension of zeolite A.

Using GC, oxalic, glyoxylic and succinic acid are determined without interference and with good reproducibility and determination limits. Fumaric and malic acid usually give reproducible results even at low concentrations, but fumaric acid is badly resolved from maleic acid and small peaks of malic acid may be difficult to distinguish from neighbouring peaks. Citric acid is prone to peak distortion in GC because of its high polarity even after derivatization. Formic, propionic and tartaric acid could not be determined with the chosen GC method.

HPLC gives very satisfactory results with most of the carboxylic acids. A very low determination limit for fumaric acid is observed whereas that for glyoxylic acid is surprisingly high. Oxalic acid suffers interference from large amounts of nitrate.

Using gradient IC, formic, propionic and citric acid can be determined with good results. Difficulties with regard to reproducibility were found with oxalic and fumaric acid, and only high concentration levels could be measured. Glyoxylic and tartaric acid

TABLE V

METHODS OF CHROMATOGRAPHIC ANALYSIS APPLIED TO THE INVESTIGATED CARBOXYLIC ACIDS

x = Suitable in most instances; (x) = suitable only in some instances; – = usually not suitable.

<i>Acid</i>	<i>HPLC</i>	<i>GC</i>	<i>IC</i>
Formic	x	–	x
Propionic	x	–	x
Citric	x	x	x
Succinic	x	x	–
Oxalic	x	x	(x)
Malic	(x)	x	–
Tartaric	x	–	(x)
Fumaric	x	x	x
Glyoxylic	–	x	(x)

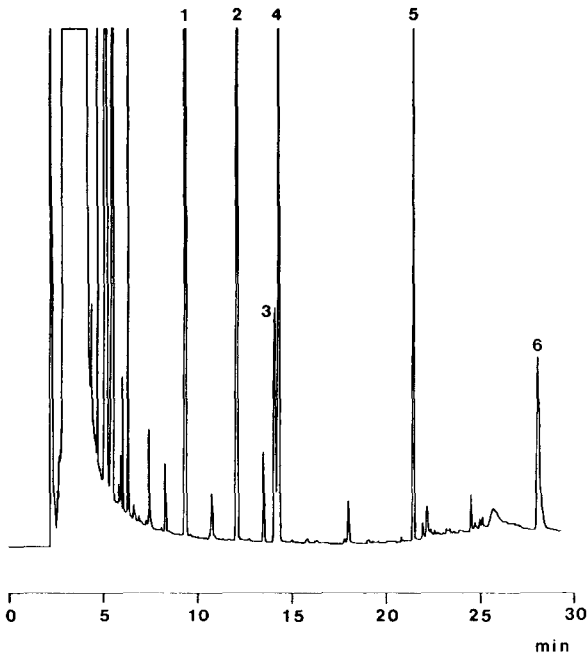


Fig. 3. Typical gas chromatogram of a derivatized extract obtained from a spiked stone sample. 1 = Oxalate; 2 = glyoxylate; 3 = fumarate; 4 = succinate; 5 = malate; 6 = citrate.

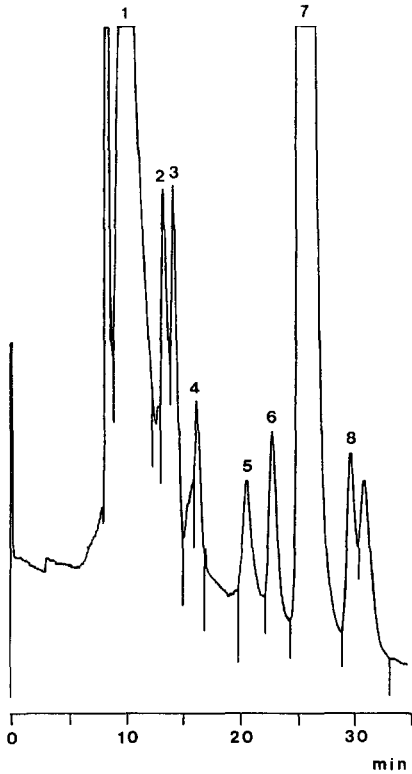


Fig. 4. Typical chromatogram obtained by HPLC from an extract of a spiked stone sample. 1 = Oxalate; 2 = citrate; 3 = tartrate; 4 = malate; 5 = succinate; 6 = formate; 7 = fumarate; 8 = propionate.

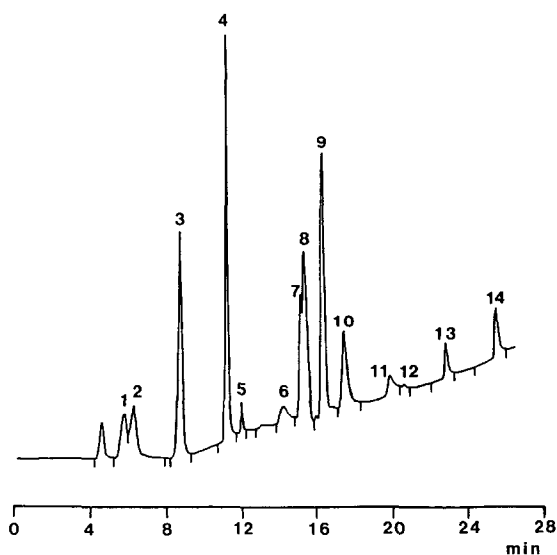


Fig. 5. Typical ion chromatogram of an extract obtained from a spiked stone sample. 1 = Acetate; 2 = propionate; 3 = formate; 4 = glyoxylate + chloride; 5 = nitrite; 6 = carbonate; 7 = malate + nitrate; 8 = succinate; 9 = tartrate + sulphate; 10 = oxalate; 11 = fumarate; 12 = phthalate; 13 = phosphate; 14 = citrate.

are subject to interference from chloride and sulphate, respectively. As succinic and malic acid coelute they cannot be determined with the chosen gradient, which was developed for the simultaneous determination of selected inorganic and organic anions²¹. Improvements in resolution may be achieved by further development.

The results presented show that the three methods employed complement each other and give the possibility of verifying results independently.

CONCLUSION

Zeolite A in an aqueous suspension is a suitable agent for the extraction of highly polar carboxylic acids from calcium-containing stones. It can be easily purified from interfering components. The recoveries and precision of the method are satisfactory down to a concentration of about $10 \mu\text{g}$ of acid per gram of stone. The described procedure has already been used to examine surface samples from some historical sites; oxalic acid is the most abundant and seems to be almost ubiquitous, but other carboxylic acids have also been found. The results should be compared with microbiological and geological findings.

ACKNOWLEDGEMENT

This research was funded by the German Federal Ministry of Research and Technology (BMFT), which is gratefully acknowledged.

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