

This article was downloaded by:

On: 18 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713640455>

The Determination of α -Keto Acids and Oxalic Acid in Rain, Fog and Mist by HPLC

Spencer Steinberg^a; Kimitaka Kawamura^a; Isaac R. Kaplan^a

^a Institute of Geophysics and Planetary Physics and Department of Earth and Space Science, University of California, Los Angeles, California, U.S.A.

To cite this Article Steinberg, Spencer , Kawamura, Kimitaka and Kaplan, Isaac R.(1985) 'The Determination of α -Keto Acids and Oxalic Acid in Rain, Fog and Mist by HPLC', *International Journal of Environmental Analytical Chemistry*, 19: 4, 251 – 260

To link to this Article: DOI: 10.1080/03067318508077036

URL: <http://dx.doi.org/10.1080/03067318508077036>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

The Determination of α -Keto Acids and Oxalic Acid in Rain, Fog and Mist by HPLC†

SPENCER STEINBERG, KIMITAKA KAWAMURA
and ISAAC R. KAPLAN

Institute of Geophysics and Planetary Physics and Department of Earth and Space Sciences, University of California, Los Angeles, California 90024 U.S.A.

(Received June 19, 1984; in final form August 22, 1984)

A simple single-step procedure for the determination of α -ketoacids and oxalic acid in rain, fog and mist is reported. The method is based upon the reversed-phase liquid chromatography of the quinoxilinol derivative of the α -ketoacids and the hydroxy-quinoxilinol of oxalic acid. This procedure is sensitive at the submicromolar level and measurements are reproducible to better than $\pm 10\%$. Oxalic acid, glyoxylic acid and pyruvic acid concentrations in fog, mist, and rain samples, collected in the Los Angeles area have been determined by the methods described in this study.

KEY WORDS: Oxalic acid, α -keto acid, quinoxilinol, reversed phase liquid chromatography, wet deposition

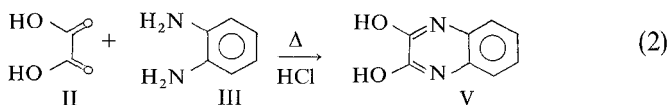
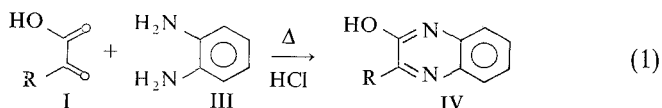
INTRODUCTION

The presence of weak organic acids in wet precipitation has recently been reported by several workers.^{1–4} Apparently, much of the organic carbon found in rain, fog and mist are in the form of low molecular weight organic acids. Recently, a standard procedure for volatile monocarboxylic acids has been reported by this group.⁴

†Contribution No. 2495: Institute of Geophysics and Planetary Physics, Los Angeles, California 90024 U.S.A.

Here, a method for determining oxalic acid and two α -ketoacids is reported. The procedure is based upon previously published techniques for the reversed-phase liquid chromatographic separation of quinoxilinol derivatives of α -ketoacids.⁵⁻⁷

α -Ketoacids (I) and oxalic acid (II) react with *o*-phenylenediamine (III), in the presence of strong mineral acids, to form the quinoxilinol (IV, Reaction 1) and the hydroxy-quinoxilinol derivative (V, Reaction 2), respectively.



Both derivatives are easily detected by ultraviolet spectroscopy or by fluorimetry. These derivatives are stable and can be separated easily by reversed-phase HPLC.

EXPERIMENTAL SECTION

Sample collection for analytical procedures

Fog and mist samples were collected at Henninger Flats, at the foot of the San Gabriel Mountains, by Global Geochemistry Corporation, Canoga Park, California by the same procedure described in Brewer *et al.* (1983).⁸ Rainwater was collected from the roof of the Geology Building at UCLA using previous methods.^{4,10}

Materials

O-phenylenediamine dihydrochloride (OPD) was purchased from Sigma Chemical Company, Saint Louis, MO and was used without purification. Water used for HPLC is deionized, distilled and then

filtered through an $0.45\ \mu$ filter. All other water was prepared by distilling "nannopure" water from alkaline KMnO_4 . 6N HCl was prepared by diluting analytical grade 12N HCl and then distilling twice in an all-glass still. NaOH and H_3PO_4 were analytical reagents from Mallinkrodt.

Preparation of derivatives

A 20 mg/ml solution of OPD is prepared in 6N HCl (ultrasound is used to promote dissolution of OPD in 6N HCl). OPD (0.25 ml) is added to 0.5 ml of sample in a screw cap test tube (with teflon-lined screw cap). The sample is then heated for 3 hours at 110°C .

After heating, the sample is processed as follows: The sample tube is cooled to 0°C , in an ice bath, and 0.15 ml of 10N NaOH and 0.10 ml of 10% H_3PO_4 are added. The pH is adjusted to 7 ± 1 by appropriate drop-wise addition of NaOH or HCl. The neutralized sample is then stored at -20°C until analysis by HPLC.

HPLC procedure

All liquid chromatography in this study was performed using an HPLC system composed of two Waters model 6000A solvent delivery modules, a Waters model U6K sample injector and a Waters model 660 solvent programmer. The column effluent was monitored at 320 nm with an Altex model 155-01 uv/vis detector. The data was recorded with a Waters data module and the peaks quantitated by height. The mobile phase consisted of a gradient mixture of 0.02 M NaH_2PO_4 (A) and acetonitrile (B). The column was a $5\ \mu\ \text{C}_{18}$ column prepared by Alltech Associates.

The column was initially equilibrated with 97:3 (A/B) at a flow rate of 1 ml/min. A 10–50 μl aliquot of sample was injected and the solvent program consisting of a 37 minute curvilinear gradient from 3% to 75% of solvent B was immediately initiated. The gradient is illustrated on the chromatogram given in Figure 1.

RESULTS

The reversed-phase separation of a standard mixture of quinoxilinol derivatives of pyruvic acid, glyoxylic acid and α -ketoglutaric acid

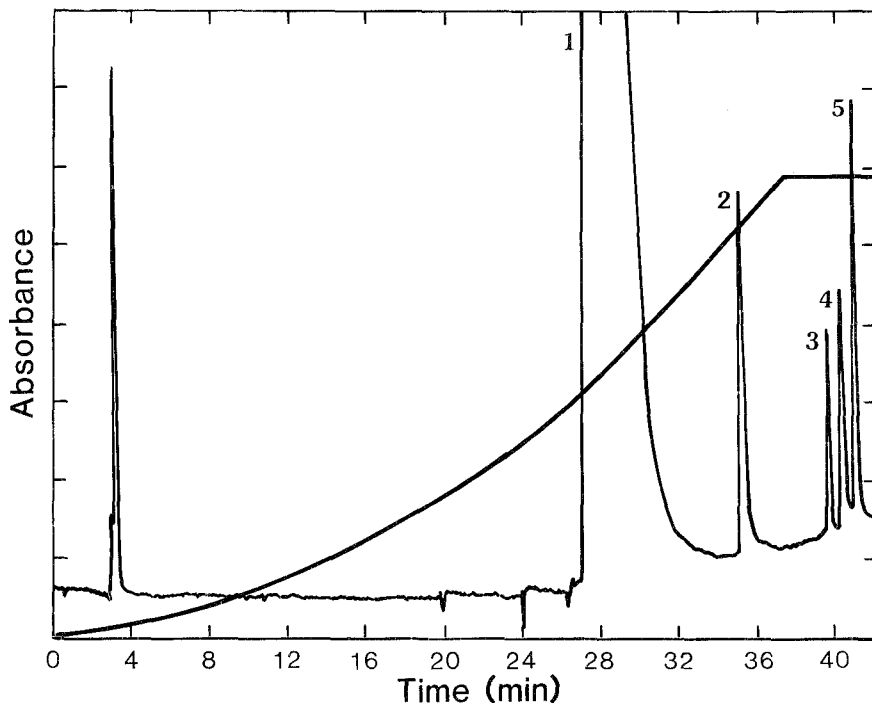


FIGURE 1 A standard chromatogram showing the reversed-phase separation of α -ketoacid and oxalic acid derivatives. The peak identifications are: (1) OPD; (2) oxalic acid, (3) glyoxylic acid, (4) α -ketoglutaric acid; (5) pyruvic acid.

and the hydroxyquinoxilinol derivative of oxalic acid is shown in Figure 1. In Figure 2, a chromatogram resulting from the analysis of a fog-water sample is shown. The peaks in Figures 1 and 2 are identified in the captions.

Variable time heating experiments at 110°C demonstrated that the extent of formation of quinoxilinol derivatives from α -ketoacids was constant after one hour of heating. Hydroxy-quinoxilinol formation from oxalate was constant after three hours at 110°C .

The linearity and reproducibility of this technique was established by standard solutions prepared with analytical grade α -ketoacids and oxalic acid. These results are presented in Figure 3. The linear fit of this data was used to establish response factors. The constancy of the response factor was rechecked each day with a standard mixture.

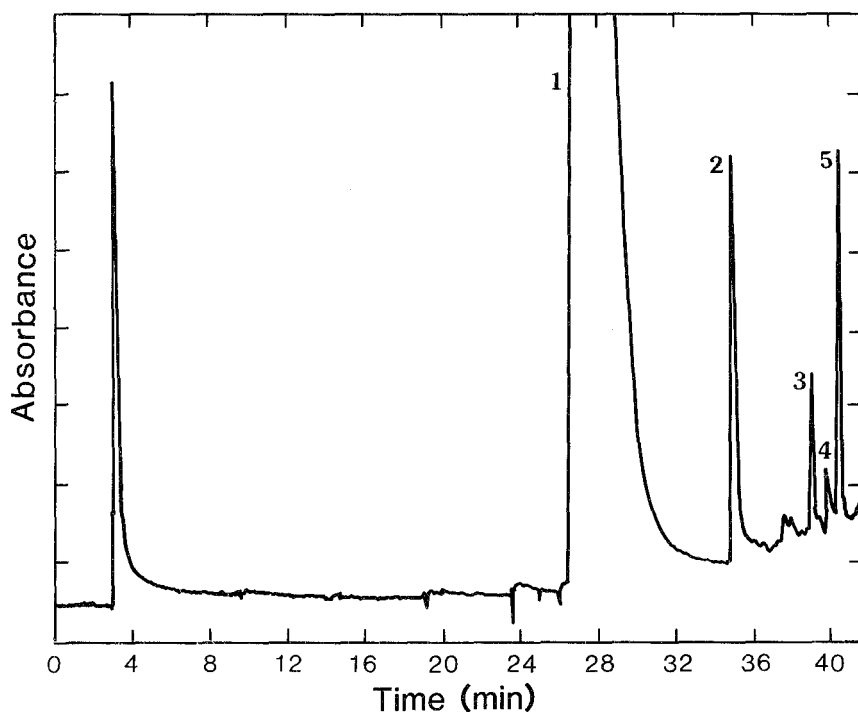


FIGURE 2 An example chromatogram resulting from analysis of a fog sample. Peak identifications are as in Figure 1.

That quinoxilinol formation from α -ketoacids is incomplete is well established.⁹ However, because the extent of derivatization is reproducible and independent of the quantity of α -keto acid present, the response factor determined can be used to correct the results for non-quantitative yields.^{5,9} The identical response factors for standard compounds and compounds in the sample solution was demonstrated by spiking a fog sample with known quantities of α -keto acids and oxalic acid. This result is illustrated in Figure 4. For a spiking experiment with oxalic acid, the slope of added oxalic acid against measured oxalic is close to 1, which demonstrates that the response factor is identical for samples and standards and is independent of the concentration of oxalic acid. Similar results are achieved with glyoxylic and pyruvic acids. Signal response for spiked

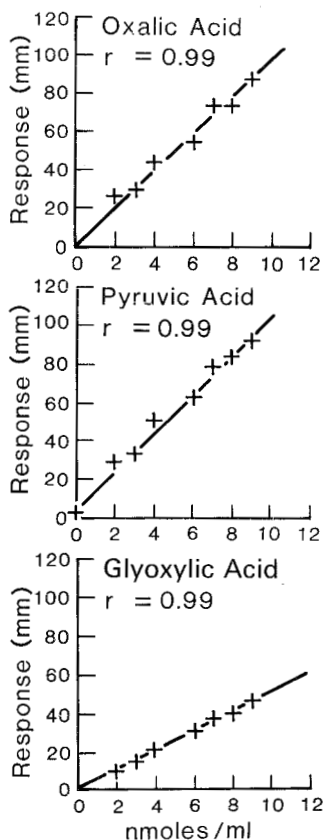


FIGURE 3 Linear correlation of oxalic acid and α -keto acid peak heights with standard concentrations.

samples and replicates determined by this technique are normally reproducible within $\pm 5\%$. The black corrected concentrations of oxalic, glyoxylic and pyruvic acids for rain, fog and mist samples collected in the Los Angeles area are presented in Table I. Because there is very little manipulation of the sample with this procedure, the sample blanks (which are determined with purified water) are highly reproducible and do not vary with sampling date. An average blank is also reported for comparison. Table I provides a representative sampling of our data. The results of additional analysis will be

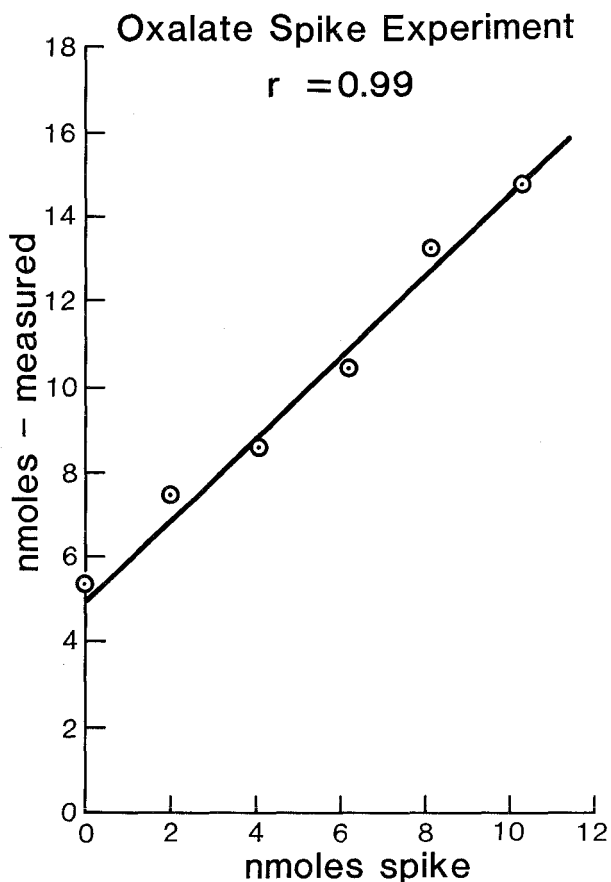


FIGURE 4 Recovery of oxalic acid added to fog water.

presented elsewhere. Several replicate determinations are also presented in Table I for two rain samples. The 9/20/83 rain sample replicate determinations (see Table I) were made five months after the initial measurement of this sample. The agreement for the replicates is better than $\pm 5\%$ for all three acids and illustrate the reproducibility of the analysis as well as the stability of oxalic and glyoxylic acids during storage. Only pyruvic acid has undergone an apparently significant change in concentration during storage at 4°C .

TABLE I

Analytical data (μM) for mist, fog, and rain collected in Los Angeles during June, 1983

Collection date		Time	Ox	Gly	Pyr
06-09-83	Mist	0630-0930	23.3	27.2	14.8
06-11-83	Mist	1515-1550	4.0	07.0	06.5
06-09-83	Fog	2352-0130	18.2	14.8	15.0
06-11-83	Fog	0440-0640	17.8	8.7	7.5
06-11-83	Fog	1051-1231	17.3	39.2	41.7
06-25-83	Fog	0637-0730	18.7	16.2	12.1
08-18-83 ^a	Rain	1030-1130	20.7	3.2	2.3
08-18-83 ^a	Rain	1030-1130	19.3	3.6	1.6
08-18-83	Rain	1730-0920	2.8	0.0	0.3
09-20-83	Rain	1530-1630	18.4	4.0	4.3
09-20-83 ^b	Rain	1530-1630	17.6	4.4	2.4
09-20-83 ^b	Rain	1530-1630	16.5	4.7	2.1
09-20-83 ^b	Rain	1530-1630	17.8	4.4	2.4
09-20-83	Rain	1630-1930	13.8	6.5	4.2
BLANK		0.7	0	0	0

^aReplicate determinations performed.

^bDetermination after 5 months' storage.

DISCUSSION

Using the above methods, we have been able to identify oxalic acid and the two α -ketoacids, pyruvic and glyoxylic acid, in rain, fog and mist samples. In addition, several samples contained low levels of α -ketoglutaric acid. Results on the concentration of oxalic, glyoxylic and pyruvic acids for several fog and mist samples collected at Henninger Flat (at the base of the San Gabriel Mountains, Pasadena, California) are presented in Table I. To our knowledge, these results are among the first published concentrations for these compounds in wet precipitation. Oxalic acid, glyoxylic and pyruvic acid range from approximately 4-30 μM . Several rain samples which were collected at UCLA were also analyzed. The rain concentrations reported in Table I, are for measurements performed on samples during the summer, when the seasonal smog conditions are most intense. The concentration of oxalic acid in these samples was comparable to fog and mist values, whereas α -ketoacid values are

considerably lower. For most samples of winter rains, oxalic acid was less than $0.5\ \mu\text{M}$ and the α -ketoacids were not measurable. Oxalic acid may be preconcentrated for analysis by adjusting 25–50 ml of a rain sample to pH 8–9 and then reducing the volume by rotary evaporation. This procedure results in the complete destruction of glyoxylic acid and pyruvic acid. Attempts to concentrate these compounds without pH adjustment results in evaporative losses in all of the acids.

At present, the sources of these compounds in fog and mist is not known. These compounds may possibly have a biogenic, as well as an anthropogenic, source. In a previous publication, we identified the α -dicarbonyls, glyoxal and pyruvaldehyde, in wet deposition.¹⁰ Possibly the α -keto acids and oxalic acids are oxidation products of the α -dicarbonyls which in turn are photooxidation products of uncombusted aromatic hydrocarbons.¹¹

Because the rain, fog and mist samples were not filtered before derivatization, it is possible that these compounds are associated with a particulate fraction, and may be liberated during sample processing. Brenner *et al.* observed oxalic acid in aerosol particles filtered from Los Angeles area air.¹²

SUMMARY

A simple method has been described for the analysis of α -keto acids (glyoxylic acid and pyruvic acids) and oxalic acid. Examples of their concentrations in urban rain, fog and mist samples from the Los Angeles area are presented. These compounds may be both biogenic and anthropogenic in origin.

Acknowledgements

We thank Robert Brewer of Global Geochemistry Corporation, Canoga Park, California for providing the fog and mist samples. This study was supported by E.P.A. (CR-807864-02-0) and DOE (DE-AS03-76SF00034, P.A. No. DE-AT03-76EV70134).

Although the information in this document has been funded wholly or in part by the United States Environmental Protection Agency under assistance agreement CR 807864-02-0 to National Center for Intermedia Transport Research, it does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

References

1. J. N. Galloway, G. E. Likens, W. L. Keene and J. M. Miller, *J. Geophys. Res.* **87**, (11), 8771 (1982).
2. G. E. Likens, E. S. Edgerton and J. N. Galloway, *Tellus* **35(B)**, 16 (1983).
3. K. W. Keene, J. M. Galloway and J. D. Holden, *J. Geophys. Res.* **88** (C9) 5122 (1983).
4. K. Kawamura and I. R. Kaplan, *Anal. Chem.* **56**, 1616 (1984).
5. S. M. Steinberg and J. L. Bada, *Mar. Chem.* **11**, 299 (1982).
6. J. F. Murray, H. W. Nolen, G. R. Gordon and J. H. Peters, *Anal. Biochem.* **121**, 301 (1982).
7. H. Hughes, L. Hagen and R. A. L. Sutton, *Anal. Biochem.* **119**, 1 (1982).
8. R. L. Brewer, R. J. Gordon, L. S. Shepard and E. L. Ellis, *Atmos. Environ.* **17** (11), 22767 (1984).
9. J. Mowbray and J. H. Ottaway, *Biochem. J.* **120**, 171 (1970).
10. S. M. Steinberg and I. R. Kaplan, *Int. J. Env. Anal. Chem.* **18**, 253 (1984).
11. D. Grosjean and P. H. McMurry, National Technical Information Service Report PB-82-262262, Springfield, VA (1982).
12. S. Brenner, W. W. Wong and L. A. Currie, (unpublished data), Global Geochemistry Corporation, Canoga Park, California.