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Comparative methodology in the determination of α-oxocarboxylates in aqueous solution Ion chromatography versus gas chromatography after oximation, extraction and esterification☆

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Abstract

The α-oxocarboxylates (α-ketocarboxylates) and the corresponding α-oxoacids (α-ketoacids) have been reported as byproducts of ozonation of potable water supplies. Some of these species also occur in biophysiological systems. Five analytes were investigated in this study: oxoethanoate (glyoxylate), 2-oxopropanoate (pyruvate), 2-oxobutanoate (2-ketobutyrate), 2-oxopentanoate (2-ketovalerate) and oxopropanedioate (ketomalonate, mesoxalate). Ion chromatography (IC) and gas chromatography (GC) were evaluated for the quantitation of these analytes at concentrations $\leq$200 ng ml^{-1}. For the IC method, the samples are run directly with minimal to no pre-treatment. For the GC method, the analytes must be derivatized with *O*-(2,3,4,5,6-pentafluorobenzyl)oxylamine to form oximes. The oximes are extracted into *tert*.-butyl methyl ether and the carboxylic acid is esterified (methylated) with diazomethane. It was concluded that the ion chromatographic determination is significantly superior to the gas chromatographic method for these analytes. Published by Elsevier Science B.V.

Keywords: Oxocarboxylates; Ketocarboxylates; Ketoacids

1. Introduction

As a class, α-oxocarboxylates can be readied for gas chromatography (GC) analysis by a two-step process [1]. First, they are derivatized with *O*-(2,3,4,5,6-pentafluorobenzyl)oxylamine (PFBOA). Second, the carboxylic acid moieties are esterified (or alternately silylated). Formation of the oxime has two advantages: (1) it permits extraction (and thus pre-concentration) of the otherwise hydrophilic ana-

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Table 1
α-Oxocarboxylates examined in this study and retention times for the methyl esters of their oximes (two geometric isomers)

Analyte anion [CAS RN]	Synonyms	Formula of acid	Retention time (min)[a]	
			Isomer 1	Isomer 2
Oxoethanoate [563-96-2][b]	Glyoxylate Formylformate	$HC(O)CO_2H$	12.28	13.14
2-Oxopropanoate [113-24-6][c]	Pyruvate 2-Methylglyoxylate	$CH_3C(O)CO_2H$	12.55	14.29
Oxopropanedioate [7346-13-6][d]	Ketomalonate Oxomalonate Mesoxalate	$HO_2CC(O)CO_2H$	19.90[g]	
2-Oxobutanoate [600-18-0][e]	2-Ketobutyrate α-Ketobutyrate	$CH_3CH_2C(O)CO_2H$	14.43	15.62
2-Oxopentanoate [13022-83-8][f]	2-Ketovalerate α-Ketovalerate	$CH_3CH_2CH_2C(O)CO_2H$	16.52	17.48

[a] Some workers have applied the (*E*)/(*Z*) nomenclature system to these geometric isomers. That notwithstanding, these compounds are not alkenes and the priority for an electron pair (on the oxime nitrogen) is not defined, although one could use the protonated form for the purpose of naming. Regardless, applying (*E*) and (*Z*) descriptors is not useful because: (1) the relative retention of the two isomers is unknown and (2) the sum of the peak areas is used for quantitation. Accordingly, the two will simply be listed in this report as isomer 1 and isomer 2, in order of elution from the column.

[b] Oxoethanoic acid monohydrate, Aldrich, Milwaukee, WI, USA.

[c] Sodium 2-oxopropanoate, Aldrich. The name 2-methylglyoxylate requires a locant of either 2 or α and should be written without a space to avoid confusion with the ester formed from glyoxylic acid and methanol.

[d] Disodium oxopropanedioate, Sigma, St. Louis, MO, USA. Although the disodium salt can be made anhydrous, both anionic forms and the acid exist as *gem*-diols at carbon 2. Thus, oxopropanedioic acid actually exists mostly in the form of dihydroxypropanedioic acid: $C(OH)_2(CO_2H)_2$. The same would be true for the deprotonated anions in aqueous solution.

[e] 2-Oxobutanoic acid, Aldrich.

[f] Sodium 2-oxopentanoate, Aldrich.

[g] Carbon 2 of oxopropanedioate is not chiral; therefore, only one geometric isomer is formed upon derivatization.

lytes into *tert*.-butyl methyl ether. (2) It introduces a functional group (C_6F_5-) that increases sensitivity by making electron-capture detection (ECD) possible. Accordingly, variations of this procedure have been used for quantitatively determining short-chain α-oxocarboxylates found as byproducts from the ozonation of potable water supplies [2–4]; however, reaction conditions are unspecified or varied between laboratories. Under the dilute concentrations (<100 μM) found in post-ozonation drinking water systems, they exist $>99.9\%$ as the ionized anions rather than the parent carboxylic acids; therefore, they can also be determined by ion chromatography (IC) [5]. These species are listed in Table 1.

The GC–ECD method continues to be relied upon for the measurement of ozonation byproducts of natural waters; therefore, it was deemed prudent to assess its ruggedness (resistance to matrix effects), reliability (day-to-day variability), and reproducibility (precision). Because this method relies on carbonyl oximation, high concentrations of aldehydes and/or ketones interfere by competing for the derivatizing agent, PFBOA, which could become a limiting reagent in waters with sufficiently high organic matter concentrations. In addition, any material that inhibits partitioning and extraction (e.g., a surfactant) can also be expected to interfere.

2. Experimental[1]

2.1. Analyte standards and test solutions

An aqueous standard was prepared at 1000 μg

[1] Mention of specific brand names or manufacturers should not be construed as an endorsement of products or companies by the United States government.

ml^{-1} in each analyte by dissolving the commercially available reagents into doubly deionized water (see Table 1); this solution was used for both the GC and IC tests. In addition, replicate standards were made from the solids on several instances and used to verify that the original standard had not deteriorated. Volumes of this stock standard were diluted 1/50 to produce a working standard 20.0 μg ml^{-1} in each analyte; this solution was confirmed to be usable for 30 days without loss of instrument response by IC. Both stock and working standards were kept in polypropylene bottles in a laboratory refrigerator at 4±1°C. The working standard was injected via microliter syringes or Eppendorf pipettor (Brinkmann, Westbury, NY, USA) into 20.0-ml portions of doubly deionized water to produce test solutions containing up to 200 ng ml^{-1} of each analyte. Blanks (no analytes added) were also prepared. Test solutions were prepared directly in pre-cleaned 40-ml glass US Environmental Protection Agency (EPA) vials with screw-caps and PTFE-lined septa obtained from Supelco, Bellefonte, PA, USA or Nalge Nunc (I-Chem), International, Rochester, NY, USA.

2.2. Assessment strategy

2.2.1. Precision

To assess the reproducibility on a single day for both the GC and IC methods, replicate standard test solutions were prepared from the stock solutions and subjected to the GC or IC method.

2.2.2. Sample holding time

Standard test solutions were prepared from the stock solutions and stored in our sample storage facility (cold room) in the dark at 7±2°C during the holding time. Subsequently, the test solutions were brought to ambient temperature and subjected to the GC or IC method.

2.2.3. Extract integrity

Although our laboratory is usually able to produce derivatized extracts of these samples within a short period, large numbers of samples which undergo GC analysis often result in a delay between the sample treatment and the instrumental analysis. Consequently, it was important to determine whether storing the extracts resulted in reduced performance. A set of extracts was run and then stored in a freezer at −15°C for 13 days to determine how much degradation occurred; this is the temperature of a typical laboratory freezer. Extracts of triplicate standards at 0 (blank), 10, 20, 50, 100 and 200 ng ml^{-1} (all analytes together) were used for this test. Additional test solutions were placed in a freezer at −80°C for up to 7 days; this temperature is the standard for sensitive biochemicals.

2.3. Gas chromatographic method

2.3.1. Oximation

Solutions of the derivatizing agent PFBOA, were prepared fresh each day at 10 mg ml^{-1} of the hydrochloride salt, PFBOA·HCl, Sigma, St. Louis, MO, USA. A 1.0-ml aliquot of PFBOA solution, and a 1.0-ml aliquot of 1.0 *M* total phosphate buffer (0.50 *M* NaH_2PO_4+0.50 *M* Na_2HPO_4) were added to each 20.0 ml standard in the 40-ml vials; salts were obtained from Fluka, Buchs, Switzerland. The vials were placed into a forced air oven thermostated at 45±2°C for 90 min. Subsequently, the vials were placed into an ice bath. After cooling, a few drops of 0.25% (w/w) FD&C Blue No. 1 aqueous solution (improves visibility of phase separation) and 1.0 ml of 9.0 *M* H_2SO_4(aq.) were added to the derivatized test solutions. This dye contains no nucleophilic moieties and thus should not interfere in the oximation. In addition, it is ionic and partitions almost exclusively into the water phase; it cannot be observed by GC–MS of the extracts. Dye was obtained from Warner Jenkinson, St. Louis, MO, USA; 18 *M* (98%, w/w) sulfuric acid was from J.T. Baker, Phillipsburg, NJ, USA. The test solutions were then extracted with a 4.0-ml aliquot of pesticide residue analysis (PRA)-grade *tert*.-butyl methyl ether (MTBE), Aldrich, Milwaukee, WI, USA. The extracts were dried with Tracepur Na_2SO_4 from EM Science, Gibbstown, NJ, USA.

2.3.2. Esterification

Methylation of the carboxylic acid functionalities was done using a flowing stream of diazomethane in argon. This minimizes the risks associated with concentrated CH_2N_2 solutions and the uncertainty associated with adding a volume of diazomethane solution to an unknown volume of recovered extract.

Solutions of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide [80-111-5] (Diazald, Aldrich) was prepared fresh prior to methylation by combining 3.0 g *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide and 30 ml of solvent (8 ml USP EtOH+22 ml PRA-grade MTBE); total volume was scaled up or down as needed. A solution of 33% (w/w) NaOH(aq.) was prepared by diluting 50% (w/w) solution obtained from Fisher Scientific, Pittsburgh, PA, USA.

The apparatus used was similar to that of EPA Method 552 [6]. Immediately preceding the methylating apparatus, the argon stream was saturated with MTBE vapor by passing it through a sintered glass dispersion tube immersed in MTBE. Extracts were transferred to 100×16 mm disposable borosilicate glass test tubes for the methylation. After 30–45 s of exposure to the gas stream, the yellow color indicated the presence of CH_2N_2; the color was used as the criterion for providing excess diazomethane. After 30 min, reaction was considered to be complete, and the methylated extracts were transferred to autosampler vials and stored at −80°C prior to GC–ECD analysis.

2.3.3. GC–ECD analysis

Derivatized and methylated extracts were analyzed on a Hewlett-Packard (Palo Alto, CA, USA) 6890 GC–ECD system equipped with an HP 7673 autoinjector. Using splitless injections, volumes of 3.0 μl were loaded onto a J&W Scientific (Folsom, CA, USA) DB-5 MS column (30 m×250 μm I.D., 0.25 μm film) at constant (high purity) helium flow of 1.0 ml min^{-1}; inlet and detector temperatures: 270°C. Temperature program: hold 60°C for 2.0 min; ramp 20.0°C min^{-1} to 120°C, hold for 1.0 min; ramp 4.0°C min^{-1} to 130°C, hold for 2.0 min; ramp 4.0°C min^{-1} to 150°C; ramp 5°C min^{-1} to 200, hold for 1.0 min; ramp 20°C min^{-1} to 260°C. Retention times are given in Table 1.

2.4. Ion chromatographic analysis

Samples were placed into 5.5-ml autosampler vials and analyzed on a Dionex (Sunnyvale, CA, USA) DX-300 ion chromatograph with conductivity detection (all parts were obtained from Dionex). We used the method of Kuo [5] without modification save instrument model; for experimental details see Table 1 of Ref. [5]. Care was taken to avoid carbonate contamination; the eluent was prepared fresh on the day of analysis from 50% (w/w) NaOH(aq.), and air exposure was minimized. We obtained similar retention times to Kuo; refer to the chromatogram in Kuo's Fig. 1 [5].

3. Results and discussion

3.1. Precision–reproducibility on a single day

As shown by Table 2, the GC method is capable of satisfactory precision under optimal conditions. Oxoethanoate and oxopropanedioate show the lowest precision, with relative standard errors of 9.3% and 7.1%, respectively, in the slopes of their calibration lines. Nonetheless, the IC method has better precision for all of the analytes except 2-oxopentanoate, which experiences chloride interference due to overlap of the two peaks. For the α-oxocarboxylates normally encountered as ozonation byproducts, analysis by IC should be more precise. The effect of the chloride peak overlap with that of 2-oxopentanoate is most pronounced at analyte concentrations below 30 ng ml^{-1}; however, our laboratory has not found this compound in ozonation byproduct formation studies. For the other analytes, agreement remains excellent (R^2>0.99) for concentrations ranging from 5 to 200 ng ml^{-1}.

3.2. Reliability–reproducibility from day to day

The ion chromatographic method far outperforms the gas chromatographic method in this area. The derivatization and methylation steps are probably responsible for the variability in the GC method. However, we do not observe such variability in the determination of aldehydes using EPA Method 556 [7]. During 9 months of testing, recoveries of quality control samples (oxoethanoate, 2-oxopropanoate, 2-oxobutanoate and oxopropanedioate) made from fresh standards have varied less than 5% for the IC method.

The most reliable results were obtained for 2-oxopropanoate, 2-oxobutanoate and 2-oxopentanoate. For these anions, the normalized (relative to injection

Table 2
Reproducibility on a single day for calibration plots (peak area vs. concentration)

Analyte	Method	Slope (ml ng^{-1})	*y*-Intercept (unitless)	R^2
Oxoethanoate	GC[a]	43±4	0[b]	0.907
	IC[c]	60 900±1400	$(-4\pm3)\cdot10^5$	0.995
2-Oxopropanoate	GC	321±7	0[b]	0.991
	IC	114 500±500	$(-6\pm10)\cdot10^4$	0.9998
2-Oxobutanoate	GC	265±6	0[b]	0.992
	IC	88 600±1200	$(-26\pm25)\cdot10^4$	0.998
2-Oxopentanoate[d]	GC	202±4	0[b]	0.992
	IC	86 000±5000	$(2\pm1)\cdot10^6$	0.974
Oxopropanedioate	GC	28±2	0[b]	0.930
	IC	117 000±1600	$(-4\pm3)\cdot10^5$	0.998

[a] Four replicates were prepared at each of the following concentrations: 0 (blank), 50, 100, 150 or 200 ng ml^{-1} (all analytes in each standard sample).

[b] Intercepts are statistically indistinct from zero.

[c] Calibration curves are based on duplicate runs of a mixed standard (containing all analytes) at concentrations of 0 (blank), 5, 10, 20, 30, 40, 50, 80, 100, 150 or 200 ng ml^{-1}.

[d] Interference from traces of chloride reduces precision and accuracy in determining 2-oxopentanoate; the chloride peak overlaps the analyte peak.

volume) least squares slopes varied less than ±10% over a 6-month period, with at least 10 sets of standards. In fact, 2-oxopentanoate is generally more reliably determined by the GC method since chloride cannot be adequately excluded from the samples and co-elutes in the IC method. Oxoethanoate quantitation was less reproducible than the others by GC. Least-squares slopes of the calibration curve could vary by as much as ±30% from day to day, without any apparent trend or reason. Nonetheless, for that day, the result was quite precise as previously indicated in Table 2 and by the error bars for the slope values in Fig. 1.

Assaying oxopropanedioate was highly unreliable by this GC method. Least-squares slopes varied by factors of 2 to 5, with some test solutions giving appropriate results and others showing no signal or less than 10% of the expected peak area. On some days, the method failed utterly for this analyte, producing data with so much scatter that no line could reasonably be drawn through a plot of peak area against concentration ($R^2<0.3$). As described in the previous section, it was possible to obtain highly precise results on some occasions; however, the method performed unsatisfactorily most of the time for oxopropanedioate.

Generally, oxoethanoate, 2-oxopropanoate and oxopropanedioate have been found in this laboratory and elsewhere as ozonation byproducts. Occasionally, we have also observed 2-oxobutanoate, but not 2-oxopentanoate[2]. It cannot be argued that degradation of the analytes is an issue because this is not observed with the IC method. In addition, there is not a steady loss of instrumental response, but rather random fluctuation.

3.3. Lower limits of detection

Data and results from the IC method permit a fairly uncomplicated estimation of the lower limits of detection (LLODs). Using the signal of the blank plus three-times the noise of the blank gives the following lower limits of detection (ng ml^{-1}): oxoethanoate 8, 2-oxopropanoate 3, 2-oxobutanoate 6, 2-oxopentanoate 60, and oxopropanedioate 7.

Calculation of LLODs for the GC method is not straightforward. Because there are so many potential chemical problems with this method, identifying the main sources of error is difficult. For 2-oxo-

[2] Although the results are not reported in this paper, we have used both the GC and IC methods for samples generated from ozonation experiments in our laboratories and from public water supplies that use ozone as a disinfectant.

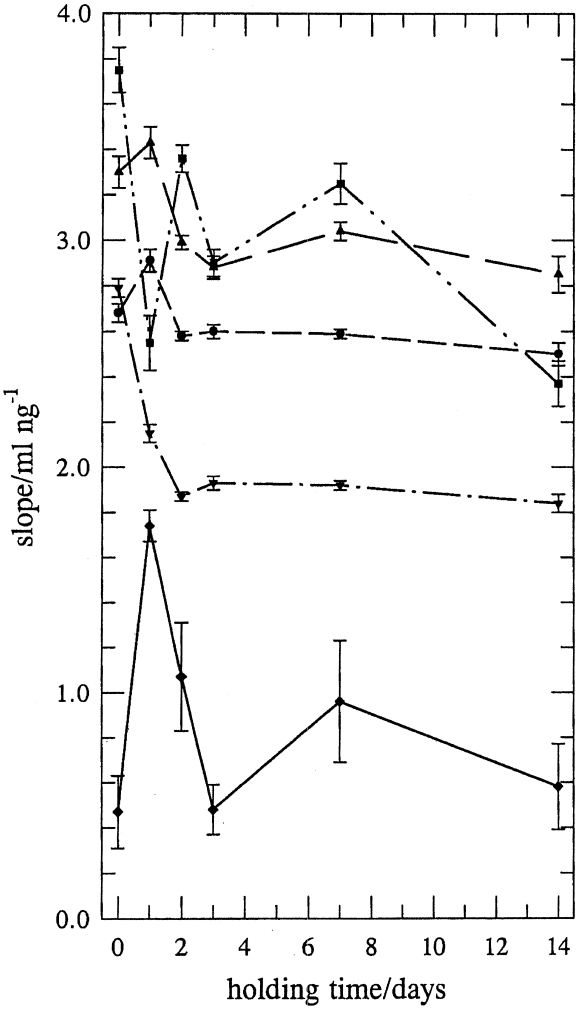

Fig. 1. Least-squares slopes of calibration lines obtained for the α-oxocarboxylates by gas chromatography. Key: oxoethanoate (■), 2-oxopropanoate (▲), 2-oxobutanoate (●), 2-oxopentanoate (▼), oxopropanedioate (♦). Much of the variability observed here is believed to result from deficiencies in method reliability rather than analyte decomposition as explained in the text.

propanoate, 2-oxobutanoate and 2-oxopentanoate, which are reliably and reproducibly measured, calibration curves are well-behaved to the 5–10 ng ml^{-1} region. Oxoethanoate achieves an LLOD of 10–15 ng ml^{-1}; however, oxopropanedioate is quite unreliable and can range from 10 ng ml^{-1} on a good day to as much as 100–200 ng ml^{-1} when method performance is poor. This is unacceptable as the concentrations measured in ozonated drinking water samples usually fall below 15 ng ml^{-1}.

3.4. Holding time

Table 3 gives the IC results for samples held at 7±2°C for a period of days subsequent to calibration with standard solutions prepared from the same stock standard; all of the solutions were made on the same day as the calibration standards. Some variation is observed, but the greatest is 23%.

The IC samples showed some loss after only 24 h; therefore, some adsorption to the container wall is suspected. Nevertheless, switching to polypropylene tubes did not alter this initial loss. Further loss may be microbially mediated rather than a simple chemical decomposition. It is worth pointing out that Kuo [5] demonstrated the stability of the analytes when preserved with benzalkonium chloride. We have continued to use mercury (II) chloride, but we do not observe a problem with quantitating 2-oxopropanoate as Kuo reported.

The slopes obtained for test solutions subjected to the GC method are highly variable from day to day, as demonstrated in Fig. 1. Based on the ion chromatography results in Table 2, we feel that much of

Table 3
Recoveries relative to day zero for standard samples held *N* days[a] and then subjected to the ion chromatographic method[b,c]

Analyte	*N* (days)		
	1	5	12
Oxoethanoate	95±3[d]	77.5±7	93±3
2-Oxopropanoate	103±2	104±1	102.5±3
2-Oxobutanoate	84±5	77±10	78±7
Oxopropanedioate	87±7	93±3	96±3

[a] *N*=0 was the starting date. *N*=1 was 24 h later, etc.

[b] Values on day 0 established a calibration curve that was used to compute the concentrations on days 1, 5, and 12 from peak areas; day 0 values were set to 100.0% recovery. Duplicate standards at 5, 10, 20, 30, 40 and 50 ng ml^{-1} were used for calibration; recoveries are based on duplicate standards at the same concentrations.

[c] 2-Oxopentanoate was omitted due to the chloride interference.

[d] Estimated standard deviation of the mean (standard error) is the uncertainty in the average: esdm=(estimated standard deviation)/$n^{1/2}$, n=6; these values were computed before rounding of the estimated standard deviations.

behavior shown in Fig. 1 is actually due to deficiencies in the GC method reliability and not analyte degradation.

3.5. Extract integrity

After 13 days at −15°C, the degradation of the methyl esters of the pentafluorobenzyloximes of the analytes was assessed by the change in the slopes of the calibration curves. Oxoethanoate (−18±10%) and oxopropanedioate (−34±5%) showed the greatest effect (loss), while 2-oxopropanoate showed a gain of questionable significance (4±3%). 2-Oxobutanoate and 2-oxopentanoate did not experience a statistically significant effect during this time. At −80°C, much of the residual water actually precipitates (particulate ice is visible), although the MTBE does not freeze. None of the analytes show a significant difference in quantitation when held at this temperature for up to 7 days. These particular times were chosen based on limitations in resources (instrumentation and personnel) for our laboratory relative to sample load. Although we did not systematically examine the stability of the esterified oximes at room temperature, we have anecdotally observed losses of up to 20% in peak area overnight. As expected, losses are greater when the laboratory is warmer. For this reason, precautionary measures such as chilling the autosampler rack and running extracts immediately are advisable.

4. Conclusion

Although the IC method does not work well for 2-oxopentanoate, this species is usually not observed as an ozonation byproduct, and we do not routinely monitor for it. For the other α-oxocarboxylates, the IC method is demonstrably better in terms of reliability and precision. In terms of practical comparative methodology, we have found the ion chromatographic analysis to be substantially superior to the multi-step GC method. The GC method requires time-consuming and potentially error-contributing derivatization, extraction, washing, methylation, and hour-long GC analysis, whereas the IC method allows multiple injections of a sample to be run directly with less volume and essentially no preparatory steps. Moreover, the IC method takes less time per analysis. Although the IC method does suffer from migrating retention times as carbonate infiltrates the eluent stock solutions, we suspect that this problem can be eliminated if the hydroxide is generated electrolytically (as with the Dionex EG-40).

Because we can collect only a limited number of samples during field studies and often cannot repeat the sampling, it is imperative that the analytical method work reliably for the three species of greatest interest: oxoethanoate, 2-oxopropanoate and oxopropanedioate (dihydroxopropanedioate). Primarily for this reason, we have abandoned the GC analysis of α-oxocarboxylates in favor of the IC analysis in studies of ozonation byproducts.

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