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Short Communication

Determination of diacetoneketogulonic acid in water samples by high-performance thin-layer chromatography

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ABSTRACT

A fast and efficient method for the determination of diacetoneketogulonic acid (DAG) —a substance relevant as contaminant in drinking water— is described. The method is characterized by enrichment of the analyte by solid-phase extraction using a innovative adsorbent and consecutive separation and quantitation by high-performance thin-layer chromatography. The method can be applied to the determination of diacetoneketogulonic acid in waste water, ground water and drinking water. The solid-phase extraction described may also be useful as sample preparation method for other separation techniques.

INTRODUCTION

Over the years, diacetoneketogulonic acid (DAG) (Fig. 1), the penultimate stage in the synthesis of ascorbic acid [1], was discharged in large amounts into surface waters by chemical manufacturers. From there the salts infiltrated the ground water. As DAG sodium salt inhibits the growth of grasses [2], the 1986 European Drinking Water Regulations restrict the concentration of this substance in drinking water to 0.1 μg/l, despite the fact that the substance has no apparent toxicity ($LD_{50_{rat,oral}}$ = 18 000 mg/kg) [3].

There are few tried and tested analytical methods capable of measuring the pesticides listed in the Regulations down to the stipulated limit of 0.1 μg/l individually and 0.5 μg/l *in toto*. The pesticides,

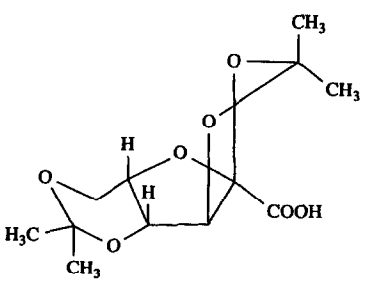

Fig. 1. Structure of DAG.

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mostly lipophilic substances, can be concentrated by solid-phase extraction on C_{18}-bonded silica gel and then determined selectively by high-performance liquid or gas chromatography.

In principle, the same procedure may be used to determine DAG. At an acidic pH DAG occurs as the free acid and can be adsorbed on C_{18}-bonded silica gel. However, in an acidic environment the substance is rapidly hydrolysed, resulting in poor recoveries.

This paper describes a method of sample preparation which avoids decomposition of DAG and provides good recoveries. Virtually any chromatographic method can then be used to separate DAG from impurities and determine it quantitatively. We chose high-performance thin-layer chromatography (HPTLC) as a rapid and cost-efficient method.

EXPERIMENTAL

Materials

All of the chemicals and TLC plates used were of "GR" or "for residue analysis" grade from E. Merck (Darmstadt, Germany). 4-Methoxybenzaldehyde was obtained from Schuchardt (Hohenbrunn, Germany) and membrane filters were Sartorius Type SM. The C_{18}-modified silica gel adsorbent was obtained from E. Merck and other suppliers, and the polymer adsorbent was Polyspher RP-18 (35 μm) from E. Merck.

Sample preparation (optimized method)

Filter the water sample over a membrane filter (only if it is not clear) and adjust the pH to 4.0 with 2.5% sulphuric acid. Pack an empty cartridge with 0.2 g of Polyspher RP-18 (35 μm) and condition the column: rinse it twice with 1 ml of ethyl acetate (leaving it to act for 10 min), twice with 1 ml of methanol (leaving it to act for 10 min) and twice with 1 ml of water (adjusted to pH 4.0 with sulphuric acid, DAG-free). Aspirate 20 ml–1 l of the sample through the column, dry the column in a stream of nitrogen for 30 min and elute with two 1-ml volumes of ethyl acetate. Add one drop of 25% ammonia solution to the eluate and evaporate it in a stream of nitrogen at a maximum temperature of 40°C to leave 0.5 ml. Apply an aliquot to the HPTLC plate. For DAG concentrations of < 5 μg/l, evaporate the eluate to leave a small drop. Apply all of this to the plate, rinse the dish with one drop of methanol and apply this to the plate also.

Thin-layer chromatography

Silica gel 60 F_{254} HPTLC precoated plates (10 × 20 cm) were used with (A) chloroform–methanol (80:20) and (B) chloroform–methanol–glacial acetic acid (80:20:2) as eluents under conditions of chamber saturation and spot or streak application. The sample volume was up to 20 μl, depending on the concentration. One-dimensional development (> 5 μg/l) was for 8 cm with eluent A, followed by drying, and then for 6.5 cm with eluent B. Two-dimensional development (< 5 μg/l) was for 8 cm with eluent A, followed by drying, turning the plate through 90°, application of a calibration series and then for 6.5 cm with eluent B.

For detection, the plates were immersed in 4-methoxybenzaldehyde solution (3 ml of 4-methoxybenzaldehyde dissolved in 80 ml of ethanol, 3 ml of concentrated sulphuric acid added and diluted to 100 ml with ethanol), heated at 130°C for 2–3 min and evaluate after 1 h. Red fluorescent spots with $R_F \approx 0.4$ were visible under long-wavelength UV light (366 nm). Chromatograms were evaluated with a TLC scanner.

RESULTS

Sample preparation

Concentration of DAG by adsorbtion on C_{18}-modified silica gel must take place at a highly acidic pH, otherwise DAG shows insufficient affinity to the adsorbent. The optimum pH is *ca.* 2. However, at this pH DAG losses due to hydrolysis are considerable, so that even with rapid operation the recovery is at best *ca.* 60% (Table I). For quantitative determinations such low recoveries require a standardized time schedule. Additionally, a standard dilution series (used to calibrate the method) has to receive the same treatment.

The use of a polymeric adsorbent with C_{18} sidechains instead of the modified silica gel does not improve recoveries significantly at pH 2. However, this material shows sufficient affinity to DAG at higher pH values, which allows adsorption at pH 4 with a recovery of virtually 100%. In comparision, the recovery at pH 4 using C_{18}-bonded silica gel is only about 40%.

TABLE I

COMPARISON OF RECOVERIES OF DAG IN DRINKING WATER: INFLUENCE OF SOLID-PHASE ADSORBENT AND SAMPLE pH

Adsorbent[a]	pH	Amount of DAG added to 500 ml of water (μg)	Amount of DAG found (μg)	Mean recovery (%)[b]
Adsorbent A, C_{18} silica gel (40–63 μm)	2	180	110	61
Adsorbent B, C_{18} silica gel (25 μm)	2	10	6.0	60
Adsorbent C, C_{18} silica gel (25 μm)	2	10	4.8	48
Adsorbent D, C_{18} silica gel (40 μm)	2	25	15.4	61
Adsorbent D, C_{18} silica gel (40 μm)	4	25	10.0	40
Adsorbent E, C_{18} polymer (35 μm)	2	25	17.1	68
Adsorbent E, C_{18} polymer (35 μm)	4	25	24.0	96

[a] Adsorbents: A = LiChroprep RP-18; B = RP-Select B; C = LiChrospher RP-18; E = Polyspher RP-18 (all from E. Merck); D = bonded-phase C_{18} (J.T. Baker).

[b] The relative standard deviation was $<5\%$ (n = 3–10) in all experiments.

During optimization, attention was given to the effect of the displacement medium on recoveries. Methanol and acetone yielded poor recoveries of *ca.* 60%. This can be improved by adding ammonia solution, although the formation of acetone condensation products and the larger number of side components adversely affect evaluation. Elution with ethyl acetate is both selective and efficient. DAG shows virtually 100% recovery and interfering impurities are mostly retained by the adsorbent. Addition of a small amount of ammonia solution prior to evaporation of the eluate has proved to be advantageous. At alkaline pH the stability of DAG is preserved.

Determination

When testing water for DAG, it is important to expect the presence of a number of substances similiar to DAG. Often seen is diacetoneketogluconic acid, a diastereomer of DAG, and diacetonesorbose. Good separations and a good limit of detection are obtained on extra-thin HPTLC pre-coated plates (Fig. 2).

Under the conditions selected, as little as 0.125 μg of DAG still produces a clear signal. This amount (obtained from 500 ml of sample) represents a concentration of 0.25 μg/l. To permit the detection of extremely small levels of DAG, the HPTLC plate must be developed two-dimensionally. DAG is thus better resolved from the interfering background,

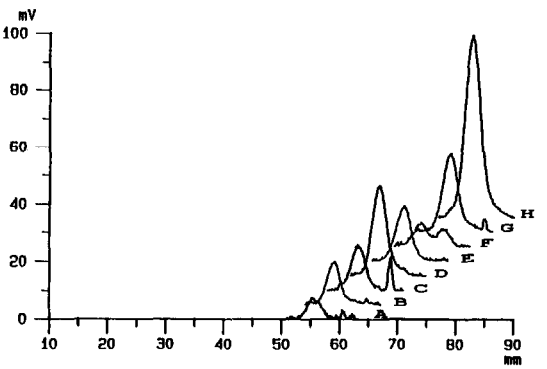

Fig. 2. Signals from a scanned HPTLC plate with calibration series from 0.25 to 2.0 μg of DAG and three samples of drinking water. Lane A, 0.25 μg of DAG; lane B, water sample containing *ca.* 1 μg/l of DAG; lane C, 0.5 μg of DAG; lane D, water sample containing *ca.* 2.5 μg/l of DAG; lane E, 0.75 μg of DAG; lane F, water sample containing *ca.* 0.5 μg/l of DAG and *ca.* 0.4 μg/l of the diastereomeric diacetoneketogluconic acid; lane G, 1.0 μg of DAG; lane H, 2.0 μg of DAG. The method is described in the text.

caused by impurities worked up concomitantly. Two-dimensional development is recommended in all instances when samples contain < 5 μg/l DAG. For higher concentrations one-dimensional development suffices.

Owing to virtually 100% recovery following work-up, a directly prepared dilution series can be used for calibration purposes. The calibration graph is linear within the range 0.125–1.5 μg, with evaluation by peak area being slightly favourable over evaluation by peak height.

At higher concentrations application must be streakwise, so as to obtain a linear signal-to-concentration ratio. By careful matching of the application and development procedures, DAG can be measured in drinking water at concentrations from 0.25 to 250 μg/l with consistent accuracy.

The method is simple and ensures both good accuracy and repeatability. This is due mainly to the fact that samples are prepared at a pH at which DAG is stable, thus ensuring complete recovery with a precision of better than 2%. Consequently, the crucial step of sample preparation is no longer critical.

DISCUSSION

The solid-phase adsorbent used for sample work-up is a polystyrene–divinylbenzene polymer with C_{18} chains on the benzene rings. In this material it seems that both the C_{18} alkyl chains and the aromatic component contribute to the adsorption, leading to a concentration effect which is superior to that obtained on C_{18}-modified silica gel. An additional advantage of this adsorbent is that it can be reused without apparent loss of activity following a simple regeneration schedule (washing with 1 *M* sulphuric acid, 1 *M* sodium hydroxide solution, water and methanol). This method of sample preparation offers significant advantages for the determination of DAG by other chromatographic methods (*e.g.*, gas chromatography–mass spectrometry following methylation) and in samples of higher concentration (*e.g.*, industrial effluents). The determination of very small concentrations of DAG is even possible with the naked eye under a long-wavelength UV lamp without apparent loss of accuracy. This makes the method especially suitable for screening purposes or for less well equipped laboratories.

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