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Measurements using gas chromatography with coelution and dual-isotope atomic emission detection

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ABSTRACT

Dual-isotope measurements by gas chromatography (GC)–atomic emission detection (AED) may enhance results for quantitative analyses. Adding a known amount of an isotopically labelled form of target analytes in each sample can compensate for irreproducibilities or uncertainties associated with sample pretreatments and sample loading. Similarly, fluctuations in AED temperatures, flows and interferants can be compensated via the added labelled forms if each target analyte and its isotopically labelled form coelute. Under these conditions they are subject to identical excitation environments and are measured from the same viewed volumes. Consequently, improved quantitative results may be attained by coelution in GC–AED methods which mimic isotope dilution.

INTRODUCTION

Atomic emission detection (AED) for gas chromatography (GC) is a powerful instrumental method which has become increasingly popular in recent years [1,2]. Multielement measurements may be made with GC–AED, providing high selectivities for a wide range of isotopes, typically spanning wide dynamic ranges with excellent sensitivities. GC–AED is usually accomplished passing eluates directly into a plasma region, typically into a microwave-induced plasma. Thus, the temporal selectivity of GC separations is supplemented by the spectral resolution of AED. Quantitative AED analyses can yield elemental concentrations for selected eluates and thereby it may be feasible to attain elemental composition and sometimes molecular formulae via GC–AED [3].

Methods using added isotopically labeled substances can be very powerful for quantitative measurements and comparisons [4–8]. Use of two or more radioactive isotopes is not uncommon and exploits the great selectivities and sensitivities of radioactivity measurements. However, potential health hazards and regulations make alternatives to use of radiolabeled materials attractive. Consequently, isotope selective methods which use non-radioactive substances, *e.g.*, mass spectrometry (MS) or atomic emission, are attractive options to radiometric procedures if appropriate isotope selectivities with sufficient corresponding sensitivities can be attained.

Dual-isotope methods with MS of equilibrated mixtures of target analytes with appropriate isotopically labeled compounds have been used for many years for accurate analyses [9]. Approaches which mimic isotope dilution and use GC–MS have gained general acceptance for use in important environmental analyses [10]. For those procedures an isotopically labeled form of each target analyte is added to samples before pretreatment and the two forms of each analyte thereby undergo essentially

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identical effects during sample preparation. Subsamples are then analyzed by GC–MS using accepted procedures, with each analyte and its isotopically labeled form being measured via their respective characteristic m/z values. However, the good selectivity and sensitivity of GC–MS is sometimes not sufficient to allow for reliable measurements via those approaches, partly due to variations in ionization efficiencies in the MS source, perhaps from variable source pressures or coeluting interferants: Ensuring coelution of both forms of each analyte can partially remedy effects of varying ionization efficiencies [8], even those caused by interferants or variable source pressures.

GC–AED is also compatible with dual-isotope techniques which mimic isotope dilution, partly because AED can concurrently measure two or more isotopes of elements from both the target analyte and its isotopically labeled form. Consequently, dual-isotope methods with GC–AED may compensate for variations in pretreatments and measurements, and ensuring coelution of both isotopic forms of target eluates can reduce variations in relative sensitivities for the two forms during the GC–AED measurements, as discussed below.

THEORY

If differently labeled forms, *e.g.*, h *vs.* d, of an analyte a elute into a microwave plasma and are atomized and excited in a thermally equilibrated region, then the observed characteristic radiant power emitted, P_E, will be related to the total number of eluate atoms, n_{ah} and n_{ad} (in atoms cm^{-3}), in the observed viewed volume, V, for form d is

$$P_{Ed} = A_{jid}(h\nu_{jid})V_d n_{jd} = A_{jid}(h\nu_{jid})V_d n_{ad} g_{jd} e^{-E_{jd}/kT}[Z(T)]^{-1} \quad (1)$$

where A_{ji} is the probability of excited state j undergoing deexcitation to state i, $(h\nu_{ji})$ is the energy of the emitted photon from the j to i transition, g_j and E_j are the statistical weight and energy for state j, $Z(T)$ is the partition function for the species, k is the Boltzmann's constant and T is the absolute temperature [11].

If both isotopic forms of the target analyte elute, then their respective radiant powers can be combined into a ratio for their corresponding j to i transition:

$$\frac{P_{Eh}}{P_{Ed}} = \frac{A_{jih}(h\nu_{jih})V_h n_{ah} g_{jh} e^{-E_h/kT}[Z(T)]^{-1}}{A_{jid}(h\nu_{jid})V_d n_{ad} g_{jd} e^{-E_d/kT}[Z(T)]^{-1}} \quad (2)$$

which, if both forms exist at the same temperature, simplifies to

$$P_{Eh}/P_{Ed} = (B_h n_{ah})(B_d n_{ad})^{-1} = (B_h B_d^{-1})(n_{ah}/n_{ad}) \quad (3)$$

where B_h and B_d are constants. Thus, their relative emission intensities from the viewed volumes would be directly proportional to the number of eluate atoms passing through the viewed volume at the time of observation.

The net measured signal for each power, $E_{measured} = E_{total} - E_{dark} - E_{background}$, will be related to the emission intensities through encoding transforms $E_m = E_{measured} = G(P_E)$, and they may be integrated over the duration of elution [11]. If the transforms are directly proportional to emission intensities, *e.g.*, $E_m = KP_E$ with K = a constant, then the relative integrated measured signals may be directly related to the respective integrated atom concentrations:

$$\frac{\int_{R-bw}^{R+bw} E_{mh}dt}{\int_{R-bw}^{R+bw} E_{md}dt} = \frac{\int K_h P_{Eh}dt}{\int K_d P_{Ed}dt} = \frac{\int K_h B_h n_{ah}dt}{\int K_d B_d n_{ad}dt} = K_h K_d^{-1} B_h B_d^{-1} \frac{\int n_{ah}dt}{\int n_{ad}dt} \quad (4)$$

where R is the analytes' retention time, w is the corresponding peak width, b is a constant, and t is time.

If mass flow patterns are constant or reproduced, then the ratio of integrated net signals should be directly proportional to the integrated concentrations of the eluates leaving the column, and thereby to the sample concentrations. Of course, non-equilibrium thermal conditions, irreproducible mass flows through viewed volumes and variable emission signal sensitivities could cause deviations from the direct relation between relative integrated net signals and relative sample analyte concentrations.

Coelution of the two forms of each analyte can force flow patterns, temperatures, etc., to be identical for the two forms even if potential interferants are present, because they would exist in the same

environment concurrently. Therefore, coelution may appreciably enhance the direct proportionality between relative concentrations and integrated areas, and thereby improve results for GC–AED analyses.

The direct proportionality between relative integrated responses, *e.g.*, chromatogram areas, and relative concentrations indicates compatibility with dual-isotope internal standard chromatography methods, for which each added internal standard is chemically identical but isotopically different than its corresponding target analyte. Moreover, the use of isotopically labeled forms of each target analyte as internal standards may also allow for: (a) possible quantitative measurements or comparisons of selected analytes without necessarily identifying each analyte [5,6], (b) for special comparisons to be made between reaction products in dual-isotope experiments [6] and (c) for effects of impurities or isotope effects to be assessed in dual-isotope reaction experiments [7].

EXPERIMENTAL

Reagents

Anthracene and decadeuteroanthracene were purchased from Aldrich, both at >99% purity. All solvents were Mallinkrodt ChromAR grade, and helium carrier gas was >99.9999% pure.

Apparatus

A Hewlett-Packard Model 5921A atomic emission detector interfaced to a Hewlett-Packard Model 5890 Series II gas chromatograph with a Model 7673 autosampler was used, controlled and monitored by a Hewlett-Packard Model 9000 computer via ChemStation software written especially for the Model 5921A GC–AED system. Emission intensities at 656.302 nm and 656.039 nm were monitored for hydrogen and deuterium, respectively. A DB-1 column, 25 m × 0.2 mm I.D. with 0.17-μm stationary phase thickness was used for all separations described herein.

Procedures

The on-column injection port was used with 1.0 μl volumes of sample for each separation and measurement. The injector was maintained at 150°C during injection, held at 150°C for 1 min then increased to 250°C at 100°C min^{-1}, maintained at 250°C for 5 min and then decreased to 200°C for the rest of each elution. Eluates were separated via a temperature program: isothermal at 150°C for 5 min and then increased at 20°C min^{-1} to 250°C. The temperature program resulted in moderate separation of the nonanthracene eluates but yielded coelution of the isotopic forms of the anthracene target analyte.

Separate solutions of natural isotopic abundance anthracene and decadeuterated anthracene were made at 0.002 *M* in methanol, *i.e.*, nearly saturated at 25°C, for these investigations. Test solutions were made by mixing and diluting the 0.002 *M* solutions, yielding solutions spanning 10^{-5} to $2 \cdot 10^{-3}$ *M* and varying in relative concentrations, $C_{\text{anthracene}}/C_{\text{decadeuteroanthracene}}$, between $5.6 \cdot 10^{-3}$ and $1.8 \cdot 10^{2}$. Triplicate replications for these solutions were analyzed by GC–AED via the temperature program described above.

RESULTS AND DISCUSSION

Moderate resolution separations via the temperature program essentially coeluted anthracene with [$^2H_{10}$]anthracene (see Fig. 1). Unfortunately, spectral resolution between deuterium and hydrogen was not complete, allowing interference into the hydrogen measurement with the presence of deuterium; thus, deuterium-caused interferences were subtracted from hydrogen results to calculate the net measured responses due to hydrogen. However, no interferences due to hydrogen were observed for deuterium even at $C_{\text{hydrogen}}/C_{\text{deuterium}}$ ratios of 180:1 and for deuterium concentrations diminishing below the limit of detection, $1.7 \cdot 10^{-11}$ mol [$^2H_{10}$]-anthracene, *i.e.*, $1.8 \cdot 10^{-10}$ mol deuterium, by the GC–AED system.

Sensitivities for both anthracene via hydrogen emission and for [$^2H_{10}$]anthracene via deuterium emission varied somewhat with concentration, as shown by curvature and slope $\neq 1.0$ in the log–log relations of their calibration plots, as expected when several orders of magnitude in concentrations are spanned (see Fig. 2). The sensitivity uncertainties were typically about ±20% relative standard deviation (R.S.D.) ($n = 3$) and may result, in part, from variations in effective plasma temperatures or variable flow patterns which fluctuate from run to run.

Relative sensitivities for the two coeluted forms, however, varied directly with their relative con-

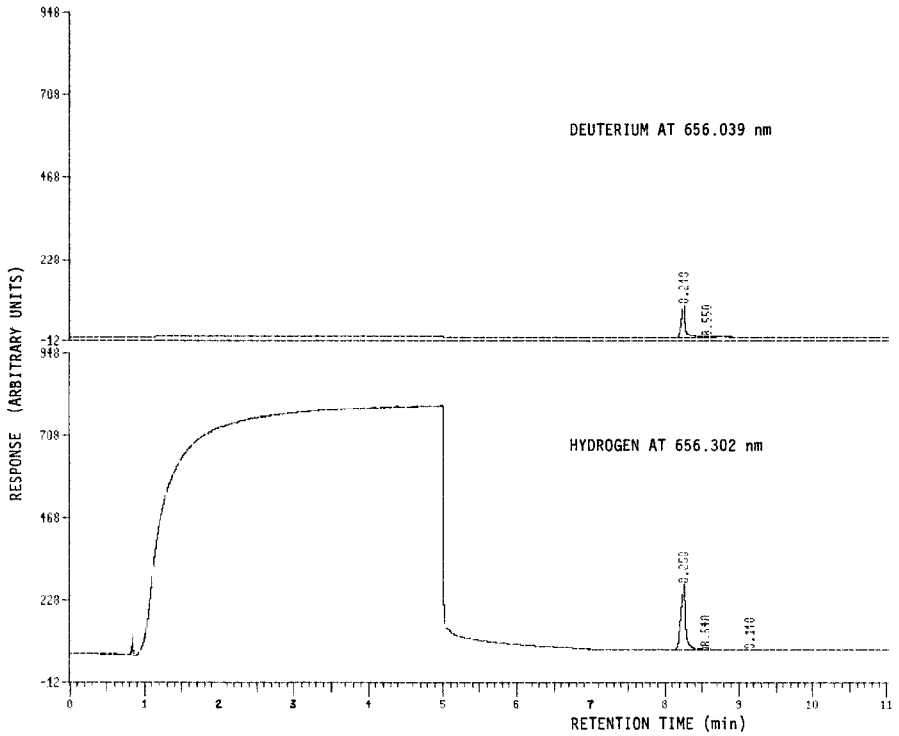

Fig. 1. GC–AED chromatograms for coelution of $3.9 \cdot 10^{-8}$ g anthracene and $3.4 \cdot 10^{-7}$ g [$^2H_{10}$]anthracene, *i.e.*, $2.2 \cdot 10^{-9}$ g hydrogen and $3.6 \cdot 10^{-8}$ g deuterium.

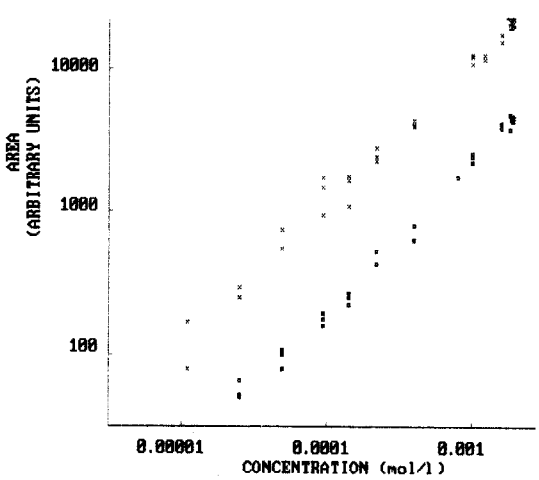

Fig. 2. Calibration plots for GC–AED measurements of anthracene (×) and [$^2H_{10}$]anthracene (□), using 1-μl injections of solutions varying in concentrations between limits of reliable measurement of about 10^{-5} mol l^{-1} to the limit of solubility of $2 \cdot 10^{-3}$ mol l^{-1}.

centrations, spanning a linear dynamic range of nearly four orders of magnitude from $C_{\text{anthracene}}/C_{\text{decadeuteroanthracene}}$ ratios of $5.6 \cdot 10^{-3}$ to $8 \cdot 10^{1}$ (see Fig. 3). Moreover, precisions for the relative sensitivities were excellent, typically varying between $\pm 3\%$ and $\pm 12\%$ R.S.D. ($n = 3$), becoming worse near the limits of detection, as expected.

Consequently, GC–AED measurements of co-eluted deuterated and normal hydrogenated anthracene is compatible with dual-isotope procedures which mimic isotope dilution. By adding a small-but-reliably-measured amount of deuterated anthracene to samples containing normal anthracene one may expect the deuterated form to work as a reliable internal and recovery standard, as well as compensating for variable excitation conditions, making more accurate and precise determinations feasible. The direct proportionality between relative sensitivities and relative concentrations is an important condition for valid use of internal standard methods

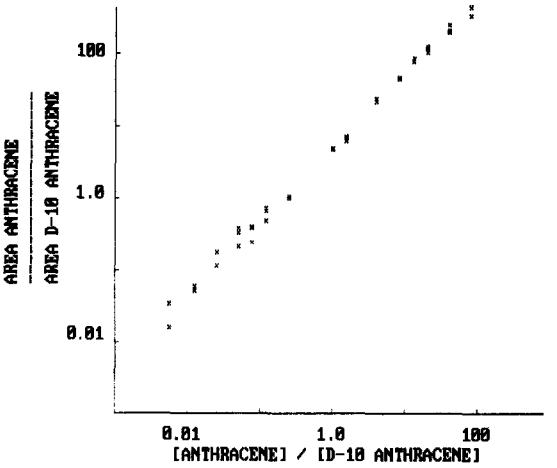

Fig. 3. Relative response *vs.* relative concentration relations for GC–AED measurements of anthracene and [$^2H_{10}$]anthracene (D-10 anthracene), using 1-μl injections of solutions varying in relative concentrations, $C_{anthracene}/C_{decadeuteroanthracene}$, between 80:1 and $5.6 \cdot 10^{-3}$:1.

for quantitative analyses with chromatography; coelution with dual-isotope internal standard procedures can be especially reliable for such determinations.

In general, GC–AED systems should be compatible with a variety of dual-isotope procedures in addition to those which mimic isotope dilution, perhaps with other compatible isotope pairs. For example, dual-isotope GC–AED methods may be useful for measurements or comparisons of reaction product concentrations [5,6] and for assessing effects of impurities or isotope effects upon reaction product formations [7]. Moreover, coelution of the isotopically different forms of target analytes may substantially compensate for variations in flows through the viewed volume and for variations in excited-state populations resulting from fluctuations in plasma conditions from run-to-run or within runs, thereby potentially improving GC–AED measurement accuracy and precisions which may be evident in higher-resolution GC separations.

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