DETERMINATIONS OF CHLORINATED HYDROCARBONS BY GAS CHROMATOGRAPHY USING RESPONSE FACTOR CALIBRATION

TERRY L. RAMUS, SCOTT J. HEIN and LAWRENCE C. THOMAS*

Oregon State University, Department of Chemistry, Corvallis, OR 97331-4003 (U.S.A.)

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SUMMARY

The response of the Hall electrolytic conductivity detector relative to a response standard may be used to determine unidentified chlorinated hydrocarbons. The detector can be used as an organic chlorine detector since the response ratio is independent of analyte structure for diverse compounds. The response standard acts as a detector response reference for all analytes determined, similar to an internal standard, but requires neither identification nor identical reference substances for each measured analyte. Response factor calibration uses Hall electrolytic conductivity detector data for chlorinated hydrocarbons, based upon a response standard, for determinations in units of moles of analyte chlorine. Additional information such as supplemental gas chromatography–mass spectrometry data allows analyte determinations in units of mass. As an example, several chlorinated hexachlorocyclopentadiene photolysis products are determined by using response factor calibration with Hall electrolytic conductivity detection.

INTRODUCTION

Quantitation procedures in chromatography generally require that pure reference substances be available from which instrumental response factors can be obtained for each analyte to be determined. Typically, determinations begin with analyte identifications which can be time consuming, or require expensive instruments and experienced technicians. Once analytes are identified, appropriate reference substances must be obtained via purchase, synthesis or purification from natural origin. Such procedures must be carried out before reliable quantitation can be assured, unless the instrumental response factor for each analyte can be obtained otherwise. Alternatively, response factor relationships are sometimes assumed. For example, in determinations by gas chromatography–mass spectrometry (GC–MS) response factors are sometimes obtained empirically for structurally related compounds[1,2], although the procedures can be invalid[1,3]. However, response factors have been obtained for GC with Hall electrolytic conductivity detection (HECD) in the absence of reliable reference substances as shown herein.

HECD is used with GC for quantitation due to its linear dynamic range over

five orders of magnitude and its sub-picogram limits of detection[4,5,6]. Selectivity for chloride by HECD in the halogen mode is greater than 10^6 relative to carbon and hydrogen[6]. Normally, analyses using HECD employ reference substances identical with each analyte[5]. However, reliable pure standards are often not available for various reasons, *e.g.*, compound instability, expense, tedious syntheses or purification complications. Despite the absence of reliable reference substances, such compounds may require determination because of their environmental persistence, suspected toxicity or involvement in important reactions. Our evaluation of the Hall electrolytic conductivity detector, operating in the reducing mode for halide detection, suggests that determinations of chlorinated organics are possible without separate response factor measurements for each analyte.

OPERATIONAL THEORY FOR THE HALL ELECTROLYTIC CONDUCTIVITY DETECTOR

GC effluents are mixed with hydrogen which then pass through the Hall electrolytic conductivity detector high-temperature nickel catalyst reactor. When an organochlorine compound contacts the catalyst, it reacts to form HCl gas which mixes with *n*-propanol solvent in a differential conductance cell[4]. The conductance of the *n*-propanol solution containing the reactor effluent minus the conductance of the *n*-propanol alone is the Hall electrolytic conductivity detector differential conductance signal, L. Normally a small baseline conductance, L_0, is observed in the absence of analyte.

N moles of a resolved chlorinated hydrocarbon, RCl_z, pass through the Hall electrolytic conductivity detector reactor and upon complete reduction Nz moles of HCl will form. As the Nz moles of HCl pass throught the Hall electrolytic conductivity detector differential conductance cell over time Δt, the total differential conductance signal, L_T, is measured. The integrated differential conductance signal, L_{RCl}, due to Nz moles of HCl is

$$L_{RCl} = \int_{t}^{t+\Delta t} (L_T - L_0)\,dt = \frac{(\lambda_{H^+} + \lambda_{Cl^-})}{l/A \cdot V_c} \cdot Nz \qquad (1)$$

where λ_{H^+} and λ_{Cl^-} are equivalent ion conductivities, V_c is the cell volume and l/A is the cell constant[7]. These terms are approximately unchanged at constant temperature and low dilutions[7,8], such that under typical detector operation conditions L_{RCl} = constant $\cdot Nz$.

L_{RCl} is therefore predicted to be proportional to Nz and independent of the specific analyte structure if catalytic degradation of organic chlorine to HCl is analyte independent. In practice, the proportionality may depend upon carrier gas and hydrogen flow-rates, furnace temperature and *n*-propanol flow-rates[6]. However, the ratio of L_{RCl} to the integrated conductance signal for a response standard will be constant if the Hall electrolytic conductivity detector conditions remain unchanged during their elution. This response ratio is related to analyte organic chlorine equivalents. Unlike traditional internal standard methods, it does not require an identical reference compound for the determination of each analyte[9]. Our assertion of analyte

independence is consistent with Lopez-Avila[5] who stated that the response of the Hall electrolytic conductivity detector in the halogen mode was roughly proportional to the number of chlorine atoms present, although deviations were observed for some p-chloroanilines and chloronitroanilines. However, these chloroanilines contain nitrogen.

The results indicate that a single calibration curve may be used for analytes which contain only carbon, hydrogen and chlorine. Response factor calibration (RFC) is demonstrated, using HECD for analyte determinations without reference substances for each analyte.

EXPERIMENTAL

Reagents

Reference substances to evaluate HECD response were purchased from Aldrich: 1-chlorooctane (1COA, 99% pure), 1,2,3-trichloropropane (TCPrA, 99 + % pure), 1,5-dichloropentane (DCPeA, 99% pure), 1-chlorobenzene (CB, 99% pure), 1,2,4-trichlorobenzene (TCB, 99 + % pure) and hexachlorobutadiene (HCBD, 98% pure). Technical grade pentachloroethane (PCEA) received from the British Drug Houses was purified by vacuum distillation to greater than 99.5% purity. Reference solutions were provided by the U.S. Environmental Protection Agency: β-hexachlorocyclohexane (β-HCCHA, 99 + % pure) and Aldrin (97 + % pure). Purity for each of the reference substances was confirmed by GC–HECD and GC–flame ionization detection. Hexachlorocyclopentadiene (HCCPD), purchased from Aldrich, was used in photolysis experiments. Resi-analyzed grade n-hexane and n-propanol solvents were purchased from J. T. Baker.

Procedures

1COA was chosen to be the response standard due to its high purity and stability. Solutions of 1COA were prepared at concentrations of $5.89 \cdot 10^3$ nmol Cl/ml, 589 nmol Cl/ml and 59 nmol Cl/ml.

The eight chlorinated hydrocarbons were divided into four subsets such that all the compounds were resolved by GC–HECD. Group I included 1COA, TCPrA and DCPeA; group II included 1COA, CB and TCB; group III included 1COA and HCBD; and group IV included 1COA, PCEA, β-HCCHA and Aldrin. β-HCCHA and Aldrin solutions were diluted from 2.5 mg/ml and 5.0 mg/ml standard solutions, respectively.

Solutions of the eight compounds were prepared as groups at eight to ten concentrations each, ranging from about 0.3 nmol Cl/ml to $1.3 \cdot 10^3$ nmol Cl/ml. 10 ml of a 1COA response standard was added to each solution prior to complete dilution. Acid-washed, hexane-rinsed glassware was used in all dilutions and storage. Class A volumetric glassware, Kirk design micropipets and n-hexane were used for all dilutions. Concentrations were calculated using known densities, molecular weights and molecular formulas[10].

A 1.7-mg/ml HCCPD solution was prepared in n-hexane. Subsamples of this solution were photolyzed under a Sylvania F15T8-BL Ultraviolet Blacklite. Following UV exposure, each subsample was diluted by a factor of 100 and evaluated in triplicate using HCCPD as an external response standard.

Chromatographic instrumentation

A Tracor Model 560 gas chromatograph equipped with a Tracor Model 700A Hall electrolytic conductivity detector was used with a 2 m × 2 mm I.D. glass column, packed with 3% OV-17 on 100/120 Supelcoport. Airco grade 4.5 helium was used as the carrier gas at 30 ml/min. Airco grade 4.5 hydrogen with a hydrocarbon trap was used as the Hall electrolytic conductivity detector reactor gas at 60 ml/min. The Hall electrolytic conductivity detector conductivity solvent, *n*-propanol, was pumped at 5 ml/min. The nickel catalyst reactor temperature was 850°C, the Hall electrolytic conductivity detector base temperature was 300°C and the GC injector temperature was 250°C. GC sample injection volumes were 1 μl. A Hewlett-Packard Model 3390A integrator was used to determine the GC peak areas and retention times.

Group I compounds were separated by a GC temperature program from 30°C to 70°C, increased at 3°C/min, after a 5-min isothermal period at 30°C. Group II compounds were resolved by a program from 30°C to 75°C at 4°C/min after a 5-min isothermal period at 30°C. Group III compounds were separated using a 3-min isothermal period at 50°C followed by temperature programming to 100°C at 4°C/min. The compounds in Group IV were resolved using a 5-min isothermal period at 30°C followed by temperature programming to 250°C at 3°C/min. The HCCPD subsamples were analyzed using a temperature program from 60°C to 200°C, at 3°C/min, after a 5-min isothermal period at 60°C.

GC–MS data was obtained on a Hewlett-Packard Model 5980A GC–MS interfaced by a membrane separator. The mass spectrometer used electron-impact ionization at 70 eV. The same GC parameters were used as described above.

RESULTS

HECD response is predicted to be proportional to the amount of organic chlorine, as discussed above. To test this prediction, responses for a variety of chlorinated hydrocarbons were evaluated with HECD. Preliminary experiments revealed that the Hall electrolytic conductivity detector sensitivity changed slowly with time, but remained constant during individual chromatograms, *e.g.*, 40 min. A response standard, 1COA, was used in these experiments to compensate for intersample changes in the detector response, variations in sample volumes, and to act as the reference substance for analyte determinations. Thus, a response ratio was defined as the analyte peak area, A_a, divided by the peak area for 1COA, A_{rs}.

Analyte concentrations ranged over four orders of magnitude from 0.3 nmol Cl/ml to 1.3 μmol Cl/ml or from 13 ng HCBD/ml to 110 μg of CB/ml. Table I shows the groupings of the eight compounds evaluated, the number of dilutions per compound, the concentration ranges, and the number of determinations.

Calibration curve

The data illustrated in Fig. 1 indicate a linear correspondence between the response ratio and the organic chlorine concentration. Curve fitting was performed by a linear least squares regression technique on the logarithmic data, as recommended when data extends over several orders of magnitude[11,12]. Log–log regression parameters and log–log parameter 90% confidence limits are given in Table I[13]. Of

TABLE I

RESPONSE FACTOR CALIBRATION DATA, LOG–LOG REGRESSION PARAMETERS AND PARAMETER 90% CONFIDENCE LIMITS FOR EIGHT CHLORINATED HYDROCARBONS

Group	Compound	n^{*}	Number of dilutions	Concn. range nmol Cl/ml	Log–log regression parameters			
					m_L^{**}	90% Conf. limits-m_L	b_L^{***}	90% Conf. limits-b_L
I	TCPrA	28	10	2.8–710	0.99	0.03	1.16	0.02
I	DCPeA	28	10	3.1–780	0.99	0.03	1.09	0.03
II	CB	45	10	3.9–980	1.01	0.03	1.24	0.01
II	TCB	45	10	0.48–1200	1.05	0.02	1.29	0.01
III	HCBD	83	10	0.28–960	1.01	0.03	1.20	0.01
IV	PCEA	34	9	2.1–1040	1.00	0.05	1.20	0.03
IV	β-HCCHA	33	9	2.6–1290	0.94	0.04	1.06	0.02
IV	Aldrin	28	8	4.1–820	0.90	0.05	1.13	0.05
All compounds		324	76	0.28–1290	1.00	0.01	1.20	0.003

 * Total number of HECD measurements for the compound.
 ** Slope of log–log regression line.
 *** Intercept for log–log regression line.
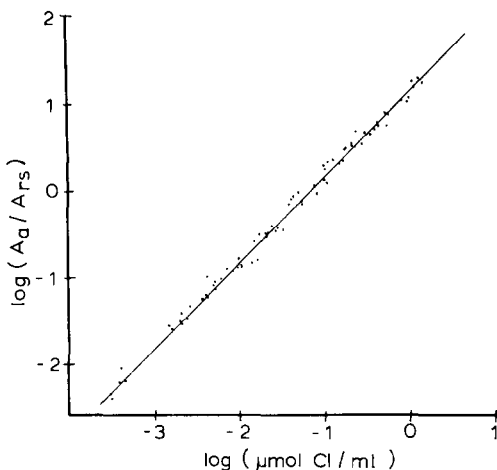
Fig. 1. Log–log plot of the response ratio vs. μmol of analyte organic chlorine. (a) points (·) represent means obtained from replicated sample injections; (b) log–log regression line (—).

331 data points, four were excluded because their concentrations were below the limit of detection, and three were deleted because of a confirmed pipeting error. The regression line calculated from the remaining 324 data points is shown in Fig. 1. Fig. 1 also contains 75 means from replicate injections of 75 different concentrations among the eight compounds tested.

These data demonstrate the linear relation between the response ratio and organic chlorine concentration since the slope of the log–log plot doesn't differ significantly from unity. Also, the response ratio did not require correction for a non-
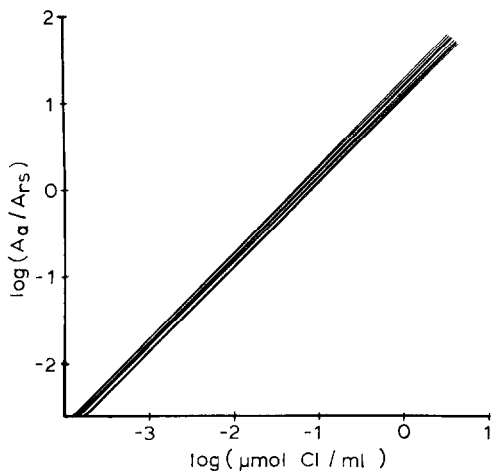
Fig. 2. Log–log regression lines for response ratio vs. μmol of analyte organic chlorine. Each line corresponds to all measurements for each of the eight compounds.

zero y-intercept prior to logarithmic curve fitting. The eight separate log–log regression lines for each of the eight compounds evaluated are shown in Fig. 2.

Photolysis product determinations

HCCPD photolysis products were determined using HECD and RFC. A 1.7-mg/ml solution of HCCPD in *n*-hexane was evaluted following exposure to UV light for 26 h. The chromatogram in Fig. 3 includes unreacted HCCPD and three unidentified photolysis products. Mass spectral data was obtained by GC–MS for the unidentified photolysis products. The molecular weight of the first product could not be determined. The second product was present below the limit of detection for our GC–MS. The number of chlorine atoms per molecule and the analyte molecular weight was determined for the third unidentified product. Analyte concentrations
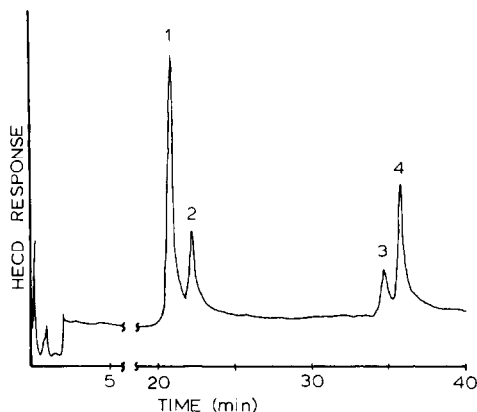

Fig. 3. HECD chromatogram of HCCPD photolysis products. 1 = HCCPD, 2, 3 and 4 unidentified HCPD photolysis products.

TABLE II

RESPONSE FACTOR CALIBRATION METHOD DATA FOR HCCPD PHOTOLYSIS PRODUCT DETERMINATIONS

NA = Not applicable.

Peak	nmol Cl/ml	Cl/molecule	nmol Analyte/ml	MW	ng Analyte/ml
1	48.0	6	7.9	272.8	2200
2*	14.0	NA**	NA**	NA**	NA**
3*	10.0	NA***	NA***	NA***	NA***
4*	26.0	8	3.2	343.7	1100

   * Analyte identity unknown.
  ** Molecular ion mass not established.
*** Analyte below limit of detection.

were determined for HCCPD and the unidentified photolysis products by RFC via an external response standard (Table II).

DISCUSSION

The utility of response factor calibration was demonstrated by using HECD. Eight chlorinated hydrocarbons with different structural characteristics all result in the same HECD response ratio calibration curve (Fig. 1). Individual log–log regression lines for each of the eight chlorinated hydrocarbons evaluated reveal small proportional errors since their parallel lines correspond to eight lines of different slopes on a linear plot (Fig. 2)[14]. Possible sources of proportional errors include: (a) minor impurities in the reference substances, (b) small changes in carrier gas flow-rate during temperature programming, and (c) inaccuracies in density values. However, log–log regression slope and intercept parameters for the eight separate regression lines could not be shown to be different by Q-tests or by null hypothesis tests based on the t-statistic at the 90% confidence level[11,15].

Response factor calibration

These studies suggest that analytes containing only carbon, hydrogen and chlorine may be determined without identical reference substances using response factor calibration. A response standard of known purity and chlorine composition must be available which is resolved from the analytes. It may be added to samples for analysis, like an internal standard, but the response standard also functions as the reference substance for all analytes determined. Alternatively, a response standard may be measured during a different elution, like an external standard, if HECD conditions remain constant.

RFC using only HECD data allows for analyte determinations in moles of organic chlorine, C_{Cl}. C_{Cl} is calculated from the response ratio, A_a/A_{rs}, the log–log calibration slope which is unity, m_L, and log–log intercept, b_L, as

$$C_{Cl} = (A_a/A_{rs}) \cdot 10^{-b_L} \tag{2}$$

If the number of chlorine atoms per molecule, N_{Cl}, can be determined via isotope ratios from the analyte mass spectrum[16], then the moles of analyte is $C_m = C_{Cl}/N_{Cl}$. If the molecular ion mass, MW, is obtained from GC–MS data, then the analyte concentration may be calculated in grams, $C_g = C_m \cdot MW$. When analyte identity is known, N_{Cl} and MW are known, and mass spectral data is not required for analyte determination in grams.

RFC requires the following for chlorinated hydrocarbon measurements with HECD: (a) the analytes and response standard are resolved, (b) the analytes do not contain elements which interfere with the HECD signal, (c) complete analyte reaction to HCl in the furnace, (d) the HECD response factor to organochlorine remains constant during a single chromatogram, and (e) differential conductance cell flow-rates remain constant during a single chromatogram.

HCCPD photolysis product analysis using RFC

RFC was demonstrated in the HCPD photolysis product experiments described above (Table II), assuming interferents were not incorporated during photolysis of HCCPD in hexane. Unreacted HCCPD was determined in ng since peak identity was known and thus N_{Cl} and MW were known. Peak 2, an unidentified photolysis product, was determined in nmol of chlorine since the analyte molecular weight could not be identified. Peak 3, another unidentified photolysis product, was determined in nmol of chlorine since the analyte was present below the GC–MS limit of detection. The last unidentified photolysis product, peak 4, was determined in ng from the molecular weight and number of chlorines per molecule obtained by GC–MS[16].

The HCCPD photolysis product measurements were accomplished rapidly, without identical reference substances for the unidentified analytes. The concentrations of HCCPD and photolysis products were also followed as a function of exposure time without time consuming identification and preparation of reference substances.

Reservations about RFC data

We have evaluated chlorinated hydrocarbons of diverse structure and have encountered no exceptions to the developed HECD calibration curve, although such compounds may exist. If exceptions are found, they might be corrected by adjusting reactor temperature[4]. Chlorinated compounds which include other substituents and Hall electrolytic conductivity detector furnace temperature effects are presently being evaluated in our laboratory.

Certainly, reservations exist for RFC determinations of unidentified analytes since these compounds may unknowingly include functional groups or elements which might cause deviations from the HECD calibration curve. However, RFC may be used for measuring unidentified analytes in the absence of reference substances in complex samples at low concentrations. Therefore, we recommend judicious use of RFC data for unknown analytes, until analyte identification or identical reference substances permit more complete study.

Application of RFC to other detection systems

Other instrumental methods might also be adapted to RFC. To use RFC, the

detection system must meet several requirements: (a) a known response *versus* concentration relationship for the measured chemical species, (b) a detector response factor for the measured chemical species which is independent of analyte origin, and (c) a constant response factor during elution of analytes and the response standard.

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