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Feasibility of Use of the Microwave Induced Plasma Atomic Emission Detector as a Compound Independent Detector for Quantitative Chromatographic Analysis

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Foi estudada a independência da resposta de um Detector por Emissão Atômica Induzida em Plasma de Microondas utilizando-se nove compostos organoclorados como amostra de teste. Com ajuste apropriado das condições operacionais do equipamento, monitorando os sinais na linha de emissão do cloro em 479,5 nm, para os nove compostos foram obtidas respostas concordantes ao nível de 8,5% ou menos, o que é adequado para uso do método de calibração independente do composto. Propõe-se que a confiabilidade da quantificação com a metodologia de calibração independente do composto pode ser melhorada, se um conjunto de compostos é usado em lugar de um único composto de calibração.

The independence of the response of a Microwave Induced Plasma Atomic Emission Detector (GC-MIP) for Gas Chromatography with the chemical structure of the analyte has been exploited using as teste samples nine organochlorine compounds. With signal monitoring at the chlorine 479.5 nm emission line and after proper adjustment of the operational conditions of the GC-MIP the agreement of the detector response for the nine compounds was of 8.5% or less, which is adequate for the use of the compound-independent calibration method. It is proposed that the reliability of the quantitation after applying the compound-independent calibration methodology may be enhanced if a set of calibration compounds is used instead of a single calibration compound.

Keywords: *microwave induced plasma, chlorine specific detection, compound-independent calibration method*

Introduction

The property of the Microwave Induced Plasma Atomic Emission Detector (GC-MIP) of behaving as a detector that may respond to analytes independently of their structures has been used in studies such as the determination of empirical formulae[1,2,3] and of quantitation by the so-called compound-independent calibration method[4,5]. In studies of this type two experimental drawbacks have to be accounted for: (a) the structure of the analyte is known, but the analyte is not available as a chromatographic standard; (b) the identity of the analyte is uncertain, but other information permits the estimation of its possible structure. In the trial of an analytical solution of the two aforementioned cases it is necessary to assume that a condition may be found under which the GC-MIP response towards the analyte is similar to its response towards a chosen calibration sample. Thus, the experimentation embodies the establishment of a criterion of assurance that the GC-MIP response towards the calibration sample and towards the analyte converge to a statistically common value. The experimentation is also not straightforward because, in general, the optimization of the performance of a GC-MIP system is not trivial as it depends on the achievement of the proper compromise among its several operational variables[6] as well as on specific characterics of the available equipment[7]. An experimental approach to establish the above-mentioned convergence of response could be based on comparison of the response of

the GC-MIP towards a set of compounds expected to be structurally similar to the sample to be quantified. These "candidate to standard" compounds should then be chromatographed under various operational conditions of the GC-MIP such as the flow-rate of the helium plasma support gas, the input microwave power and the injected mass of the element of choice for detection. Evaluation of the response data of the GC-MIP for the candidate for standard compounds would reveal if it is possible to assume that the GC-MIP response towards these compounds converges to a common value within an assumed uncertainty. Then the quantitation of the analyte would be undertaken by interpolation from the response data of the calibration set. One approach to this experimental procedure is described in the present paper. Here, nine analytes were used in the search for conditions that could lead to the convergence of response of a GC-MIP at the 479.5 nm chlorine emission line. As will be shown this experimental procedure represents a systematic approach that can be used to ascertain if a non-structural dependence of the GC-MIP response may be assumed and at which level of uncertainty.

Materials and Methods

GC-MIP system (prototype)

Gas chromatograph: model HP-5890A (Hewlett-Packard, Avondale, PA); chromatographic capillary column: Econo-Cap (Alltech, Deerfield, IL) SE-30 column (L = 30 m, d_i = 0,25 mm, d_f = 0,25 μm). Plasma generation: 2.45 MHz microwave power generator GMW-303-DR (AHF Analysentechnik, Tubingen, Germany) connected to an Enhanced Beenaker-type resonant cavity (AHF Analysentechnik) through a 50 Ω coaxial cable; discharge cell: 5 mm o.d., 3 mm i.d. quartz tube, connected to the cavity through a brass adapter; chromatograph-to-cavity transfer line: 1/8" i.d. insulated copper tube, directly heated by the GC oven; plasma support gas: analytical grade helium. The emission from the plasma was focused by a f = 150 mm fused-silica lens onto the 0.1 mm entrance slit of a model Multispec 125 mm monochromator (Oriel, Stratford, CT). The signal amplification was accomplished with a model 77344 photomultiplier tube connected through a lab-made pre-amplifier to a model 7070 photometer readout (Oriel). The amplified signal was digitized by a model DT-2801A ADC (Data Translation, Marlboro, MA) connected to a 40 MHz 486-DLC PC-compatible microcomputer. The data analysis was accomplished with the program ANACROM[8], written in our laboratory using the Turbo Pascal 5.0 compiler (Borland Corp., Scotts Valley, CA)

Reagents

Isooctane (Aldrich, Milwaukee, WI); 1-bromo-3-chloropropane (Shell Chimie, Paris, France); chloroform, benzene, 1,2-dichloroethane and tetrachloroethylene (Reagen,

Rio de Janeiro, Brazil). 1-chlorobutane o-dichlorobenzene and chlorobenzene (Vetec, Rio de Janeiro, Brazil); p-chlorotoluene and hexachloroethane (Carlo Erba, Milan, Italy). All reagents were of analytical grade.

Experimental

The studies aimed to establish the behavior of the GC-MIP response towards organochlorine compounds with signal monitoring at the chlorine 479.5 nm emission line. Thus, to warrant the higher possible reliability of the Cl signal an experimental procedure was undertaken to establish the operational conditions of the GC-MIP that enhanced the molar chlorine to carbon selectivity (Cl/C)[9] at the Cl emission line. In this procedure chloroform and n-hexane dissolved in isooctane were used as test compounds. The tests were carried out as follows.

• *Chromatograph:* carrier gas: He at 0.8 mL min^{-1}; column oven temperature: 50 °C to 70 °C at 5 °C min^{-1} ; injector temperature: 180 °C; injected sample volume: 0.2 μL; split ratio: 1/95.

• *Plasma Generation:* Helium support gas flow (F) in the range of 231 mL min^{-1} to 1501 mL min^{-1}; microwave input powers (P) of 60 W, 80 W and 100 W.

• *Sample amount:* The concentrations of $CHCl_3$ and of C_6H_6 were adjusted for the detection of, respectively, 8 ng of chlorine and 135 ng of carbon.

After the establishment of the MIP conditions that resulted in the hightest Cl/C value, the experiments with the isooctane solutions of the mixture of the aforementioned nine organochlorine compounds were carried out under the following conditions.

• *Chromatograph:* carrier gas: He at 0.8 mL min^{-1} ; column oven temperature: 45 °C (3 min), to 55 °C at 2.5 °C min^{-1}, from 55 °C to 120 °C at 25°C.min^{-1} up, held at 120 °C for 5 min; injector temperature: 250 °C; injection volume: 0.2 μL; split ratio: variable from 1/95 to 1/105.

• *Plasma Generation:* Helium support gas at 231 mL min^{-1}, 654 mL min^{-1} and 1501 mL min^{-1}; microwave input power at 60 W and 100 W.

• *Sample amount.* Two solutions of the nine organochlorine compounds in isooctane were prepared to allow the detection of respectively 8 ng and 50 ng of chlorine for each analyte.

In the chromatographic analysis of the nine organochlorine compounds all injections were performed in triplicate. For each chromatographic peak the ratio of the peak area to the injected mass (A/m) of Cl was calculated. Tetrachloroethene was arbitrarily elected as a reference compound and the A/m of each of the other compounds were divided by the A/m of tetrachloroethene. The ratios of A/m were the parameters used in the calculation of the convergence of response. The ratios of A/m are normalized

responses, herein indicated as RN_i were **i** stands for each of the studied compounds.

Results and Discussion

The variation of the Cl/C molar selectivity at the Cl 479.5 nm emission line was accessed after using three levels of microwave input power and six levels of helium flow. The obtained Cl/C (average of triplicates) are shown in Table 1 together with their percent standard deviation (s_R).

From Table 1 it can be ascertained that Cl/C increases in direct proportion to the F_{He}, for each P. The ultimate cases of high Cl/C are indicated with asterisks in Table 1, which were considered as being virtually infinite Cl/C because while the signal for chloroform increased as F_{He} was increased, the benzene signal was not distinguishable from the chromatographic baseline. These results suggested that the achievement of the most reliable and pure Cl signal at the 479.5 nm emission line could occur with the MIP operated with P = 100 W and F = 1501 mL min^{-1}. It was also presumed that the independence of the response of the GC-MIP with the chemical structure of the analyte would probably occur at the MIP operational condition that ensures the purest Cl signal. Nevertheless, in the subsequent studies of convergence of response for the nine studied compounds other operational conditions were also tested for comparative purposes. Twelve GC-MIP operational conditions were used, a combination of two levels of P, three levels of F_{He} and two levels of detected Cl mass. For each GC-MIP operational condition triplicate chromatograms of the nine compounds were obtained, the average normalized response RN_i was calculated for each compound and the obtained set of nine RN_i were fitted through regression analysis to the surface given by Eq. 1.

$$RN = a_1 + (a_2 t'_R) + (a_3 C) + (a_4 H) + (a_5 + Cl) +$$
$$+ (a_6 C H) + (a_7 H Cl) + (a_8 C Cl) \qquad (1)$$

As can be deduced from its inspection, Eq. 1 relates the RN_i to the following parameters of the studied compounds: the adjusted retention time (t'_{Ri}), the number of carbon, hydrogen and chlorine atoms per formula (C_i, H_i, Cl_i) and to the paired product of the atom numbers, *i.e.* $C_i \bullet H_i$, $C_i \bullet Cl_i$

and $H_i \bullet Cl_i$. For each of the used GC-MIP operational conditions Equation 1 is defined by the regression coefficients a_1 to a_8, which were obtained after submitting the correspondent RN_i to regression analysis using the Excel 5.0 spreadsheet (Microsoft Corporation, Redmond, WA). Then, each form of Eq. 1 was used to calculate, for each studied compound, the interpolated normalized response ($<RN_i>$) and the percent residual ($R_{s\%}$) - the difference between $<RN_i>$ and RN_i relative to $<RN_i>$. Table 2 displays the RN_i, the $<RN_i>$ and the $R_{s\%}$ obtained for the nine compounds under the condition of P = 100 W, F_{He} = 100 mL min^{-1} and detected Cl mass of 8 ng. The RN_i of Table 2 were calculated from chromatograms similar to the chromatogram depicted in Fig. 1. Although the $<RN_i>$ and $R_{s\%}$ for the other studied GC-MIP operational conditions were also calculated their listing was not included here because it would be too extensive. Moreover, it will be clarified in the following discussion that the $<RN_i>$ and $R_{s\%}$ of Table 2 are the most relevant for the present discussion.
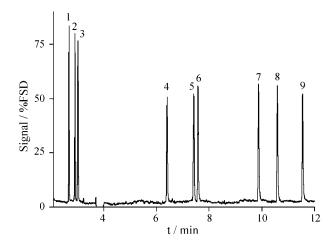

Figure 1. Typical chromatogram obtained for the mixture of nine compounds under the MIP conditions of P = 100 watts and F_{He} = 1501 mL min^{-1}. In their order of elution the analytes are: 1 = chloroform, 2 = 1,2-dichloroethane, 3 = 1-chlorobutane, 4 = tetrachloroethylene, 5 = 1-bromo-3-chloropropane, 6 = chlorobenzene, 7 = p-chlorotoluene, 8 = o-dichlorobenzene, 9 = hexachloroethane. The chromatographic conditions are described in the Experimental.

Table 1. Chlorine to Carbon Selectivities[a] Obtained at the 479.5 nm Emission Line under Varied Helium Flow and Microwave Input Powers.

F_{He} / mL min^{-1}	231	428	654	908	1191	1501
60 W	20 (5.5)	41 (2.2)	76 (3.1)	90 (9.3)	91 (6.8)	154 (19.0)
80 W	26 (4.0)	44 (1.0)	80 (3.0)	102 (9.0)	104 (10.0)	*
100 W	31 (4.0)	46 (3.0)	78 (4.0)	*	*	*

[a] The s_r are shown between parenthesis below the corresponding average Cl/C value.

Table 2. Results of the surface fit after Eq. 1 for the RN data obtained at P = 100 W, F_{He} = 1559 mL min^{-1} and 8 ng of detected Cl. The adjusted retention times, t'R, are shown for illustrative purposes.

Compound	t_R'	RNi [a]	$<RN_i>$ [b]	$R_{s\%}$
CHCl$_3$	1.39	0.863	0.937	8.5
C$_2$H$_4$Cl$_2$	1.67	1.009	0.935	-7.3
C$_4$H$_9$Cl	1.81	0.846	0.872	3.0
C$_2$Cl$_4$	5.96	0.985	0.930	-5.6
C$_3$H$_6$BrCl	7.20	1.014	1.019	0.5
C$_6$H$_5$Cl	7.40	0.927	0.939	1.3
C$_7$H$_7$Cl	10.12	1.000	0.964	-3.6
C$_6$H$_4$Cl$_2$	10.95	0.927	0.964	4.0
C$_2$Cl$_6$	12.08	0.986	0.997	1.1

[a, b] see text for explanation.

The values of the $R_{s\%}$ shown in Table 2 indicate that, for the condition of P = 100 W, F_{He} = 100 mL min^{-1} and Cl detected a mass of 8 ng, the approach of fit of the RN_i data to Eq. 1 permits interpolation of each of the nine $<RN_i>$ with a level of bias that is not greater than 8.5% and that for most (seven) of the studied compounds remained below 6%. Nevertheless, the data of Table 2 do not indicate if the $<RN_i>$ tend to a common value, *i.e.* if the searched convergence of response among the studied compounds was attained. To assess the convergence of response the nine $<RN_i>$ of Table 2 were paired and compared. For this comparison the absolute percent differences (|APR|) between the $<RN_i>$ were calculated according to Eq. 2,

$$| APR| = 100 \, abs \, [\frac{(<RN_{i=Z}> - <RN_{i=Y}>)}{<RN_{i=Z}>}] \qquad (2)$$

where $<RN_{i=Y}>$ and $<RN_{i=Z}>$ are the general representation of the $<RN_i>$ for the 36 possible pairs of com-

pounds used as test samples - *e.g.* Z and Y could be chlorobenzene and hexachloroethane, etc. The |APR| may be taken as the analytical error (bias) to be expected whenever the quantitation of a compound Z is calculated using compound Y as the analytical standard. Thus, as indicated by the data presented in Table 3, the |APR| may reveal the possibility for the convergence of response.

After analyzing Table 3 for the data of the nine compounds it can be seen that out of 36 possible comparisons, 34 pairs of compounds fall within 10% of bias between the respective $<RN_i>$. This conclusion applies for the chromatographic data obtained using the GC-MIP conditions of P = 100 W, F_{He} = 100 mL min^{-1} and detected Cl mass of 8 ng. This same type of data evaluation was carried out using the |APR| that corresponded to the other studied MIP operational conditions. As is shown in Table 4, it was found that the MIP operational condition of P = 100 W, F_{He} = 100 mL min^{-1} and detected Cl mass of 8 ng resulted in a greater number of pairs of compounds with less than 10% of bias between the respective $<RN_i>$.

The above reasoning indicates that for the herein studied test sample the convergence of response occurs at the 479.5 nm Cl emission line when the GC-MIP operational condition favors a high chlorine to carbon selectivity. Nevertheless, the data of Table 3 may be further exploited as, within its set of nine compounds, subgroups may also be

Table 4. Number of pairs of compounds that present 10% or less of bias observed at different combinations of Helium flow (FHe) , microwave input power and detected mass of Cl.

	mCl = 50 ng		mCl = 8 ng	
F_{He} / mL min^{-1}	60 W	100 W	60 W	100 W
216	21	22	24	30
641	14	22	16	18
1501	6	5	25	34

Table 3. Absolute Percent Difference (|APR|) between the pairs of the studied compounds obtained at P = 100 W, F_{He} = 1559 mL min^{-1} and 8 ng of detected Cl.

	$<RN_i>$→↓	CHCl$_3$	C$_2$H$_4$Cl$_2$	C$_4$H$_9$Cl	C$_2$Cl$_4$	C$_3$H$_6$BrCl	C$_6$H$_5$Cl	C$_7$H$_7$Cl	C$_6$H$_4$Cl$_2$	C$_2$Cl$_6$
		0.94	0.94	0.87	0.93	1.02	0.94	0.96	0.96	1.00
CHCl$_3$	0.94	-	0.2	7.5	0.8	8.0	0.2	2.8	2.8	6.0
C$_2$H$_4$Cl$_2$	0.94		-	7.3	0.6	8.2	0.4	3.0	3.0	6.2
C$_4$H$_9$Cl	0.87			-	6.3	14.4	7.2	9.6	9.6	12.6
C$_2$Cl$_4$	0.93				-	8.7	1.0	3.6	3.5	6.7
C$_3$H$_6$BrCl	1.02					-	8.5	5.7	5.7	2.2
C$_6$H$_5$Cl	0.94						-	2.6	2.6	5.8
C$_7$H$_7$Cl	0.96							-	0.0	3.3
C$_6$H$_4$Cl$_2$	0.96								-	3.3
C$_2$Cl$_6$	1.00									-

considered. One subgroup is that formed by the aliphatic compounds $CHCl_3$, $C_2H_4Cl_2$, C_2Cl_4 and C_2Cl_6 that could be chosen as "candidate for standard" compounds for the analysis of a chlorinated aliphatic analyte expected to have one or two carbon atoms in its structure. The inspection of the data of Table 3 for $CHCl_3$, $C_2H_4Cl_2$, C_2Cl_4 and C_2Cl_6 reveals that it would be reasonable to expect no more than 6% of bias in the quantitation of such an analyte using the response data of any of the four compounds. It is also evident from Table 3 that the three aromatic compounds, C_6H_5Cl, $C_6H_4Cl_2$ and C_7H_7Cl also correlate very well, as their |APR| are 2.6% or less. These levels of expected error of <6% and <2% should suffice for quantitation by the compound-independent calibration method[4] and for studies of empirical formulae because data such as retention times and the values of specific emission lines could be cross-correlated to ascertain the proper identification of the analyte under study[2]. The data of Table 3 may also indicate possible outliers in the set of standard compounds, as would be the case of using C_3H_6BrCl and the four above-mentioned aliphatic compounds as the set of standards. After inspecting the data of Table 3 it is noted that the |APR| between C_3H_6BrCl and any of the other aliphatic compounds is more than 8.2% - with an extreme case of 14.4% - which strongly indicates that C_3H_6BrCl should be eliminated from the set. Thus, the comparison of the |APR| may also reveal outliers in the test sample.

Conclusions

The possibility of undertaking quantitative analysis without the need for the specific target compound to construct calibration curves is a very useful feature when it is hard to find such a compound in a "pure" state. The approach proposed in this work permits a systematic evaluation to establish the independence of response of the GC-MIP detector to the chemical structure of the analyte. In the present study it was important to establish the GC-MIP conditions that permitted the acquisition of essentially "pure" chlorine signals, which seems to be imperative for the purpose of achieving the desired convergence of response among the chlorinated analytes. In this evaluation it was found that when working with GC-MIP operational conditions that enhance the chlorine to carbon selectivity, nine compounds of very varied structure tend to show the same response at the chlorine emission line. Thus, it may be concluded that quantitative procedures based on data interpolation through the compound-independent calibration method are potentially feasible by using the property of elemental selectivity of the GC-MIP.

Acknowledgments

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