

# Experimental Proofs of a Correlation Between the Coefficients for the Slope of the Response Line and the Response Factor of an ELSD

Sylvie Héron,<sup>1</sup> Marie-Geneviève Maloumbi,<sup>1</sup> Michel Dreux,<sup>2</sup> Eric Verette<sup>2</sup> and Alain Tchaplà,<sup>1</sup>

<sup>1</sup>Groupe de Chimie Analytique de Paris-Sud. EA 4041, LETIAM — IUT d'Orsay, Orsay, France

<sup>2</sup>SEDERE, Alfortville, France.

Evaporative light-scattering detection (ELSD) is becoming more popular in liquid chromatography (LC) because it is compatible with gradient elution and can detect compounds that do not contain a UV chromophore. The light-scattering phenomenon is complex and may result from different contributions of Rayleigh, Mie and refraction–reflection according to the ratio of the scattering particle diameter (the scatterer) and the wavelength of the incident beam of a light source.

The measured peak area ( $A$ ) is correlated to the analyte mass ( $m$ ) according to the empirical relationship  $A = a \cdot m^b$ , where  $b$  is the slope of the response line and  $a$  is the response factor. A study was undertaken to evaluate the correlation between  $b$  and  $a$  values because they are regarded as independent numerical coefficients. For a given detector, a linear correlation between  $b$  and  $\log a$  has been observed.

Basically, the response of a chromatographic detector is typically evaluated from the peak area of eluting species. This area ( $A$ ) can be expressed as the integral of responses over the elution time interval from the beginning ( $t_i$ ) to the end ( $t_f$ ) of the chromatographic peak:

$$A = \int_{t_i}^{t_f} a \cdot S(t) \cdot dt \quad [1]$$

where  $S(t)$  is the instantaneous detector response at time  $t$ , and  $a$  the response factor.<sup>1</sup>

The integration of the equation performed over a Gaussian

peak shape leads to the well known quantitative relationship between the area of the chromatographic peak and the analysed solute quantity.

For a concentration or a mass sensitive detector, assuming a response factor is constant over a particular range of analyte concentration, the integration leads to the equation:

$$A = a \cdot m \quad [2]$$

where  $A$  is the peak area,  $a$  is the response factor and  $m$  is the sample mass.

The most important specification of an LC detector is its

linearity making the quantitative analysis easier. Most detector manufacturers claim a linear response over a particular concentration range for any specific detector but, in fact, linearity is a theoretical concept and can only be approached, and rarely, if ever, reached.<sup>2</sup> Because of this, Fowliss and Scott proposed a means of measuring linearity that can be defined by the following equation:<sup>3</sup>

$$y = a \cdot c^b \quad [3]$$

where  $y$  is the output signal from the detector,  $a$  is the response factor,  $c$  is the solute concentration and  $b$  is the response index.

After integration Equation 3 is equivalent to

$$A = a \cdot m^b \quad [4]$$

For a real linear detector, the response index of the detector ( $b$ ) should be equal to unity and the proximity of  $b$  towards unity gives a measure of the linearity. According to Scott,<sup>4</sup> no detector has been manufactured so far that has a  $b$  value equal to unity over more than two decades of concentration range. However, regarding quantitative accuracy, for a  $b$  value higher than unity, the higher the value the more accurate the quantitative analysis becomes.

In LC, most of the detectors (UV, fluorescence etc.) give linear responses for analytes injected at diluted solutions except for evaporative light-scattering detectors (ELSDs).

To simplify, when using an ELSD, Mourey and Oppenheimer have approximated the peak shape as a square wave with an average instantaneous signal  $\bar{S}(t)$ .<sup>5</sup>

$$A \approx \bar{S}(t_2 - t_1) \quad [5]$$

This approximation is valid if the instantaneous response  $S(t)$  per unit sample concentration is fairly constant. When the experiments are performed at a constant eluent flow-rate, if the peak interval  $t_2 - t_1$  remains constant, the effects of sample and solvent properties can be directly related to changes in  $\bar{S}$ .

Moreover, the authors showed that the average instantaneous signal for a collection of particles is given by

$$\bar{S} = Nf \int_{\lambda_1}^{\lambda_2} \int_{X_1}^{X_2} I(\theta, \lambda, X) \cdot P(X, t) \cdot f(\lambda) dX d\lambda \quad [6]$$

where  $Nf$  is a constant number of particles, which are generated from the evaporation of a constant number of droplets;  $I(\theta, \lambda, X)$  is the scattering intensity of an individual particle at an angle of observation  $\theta$ , with incident light of wavelength  $\lambda$ ,  $f(\lambda)$  is the spectral response of the optical system and  $P(X, t)$  the probability of occurrence for a given particle size.<sup>5</sup>

As the scattered light intensity ( $I$ ) for a given  $\lambda$  and  $\theta$  is related to the size of particles,<sup>5-8</sup> this leads to the empirical equation  $A = a \cdot m^b$  valid for quantitative ELS detection, where  $b$  is related to the size of particles that scatter the light and  $a$  to the  $Nf$  value.

Mourey and Oppenheimer have also demonstrated that the velocity at which particles reach the photometer will proportionally increase or decrease with their volumetric dilution in the nebulizing gas stream,<sup>5</sup> which leads to a change

## The most important specification of an LC detector is its linearity making the quantitative analysis easier.

in the total number of particles passing through the detector per unit of time ( $Nf$ ). Such a dilution can be directly related to the experimental conditions of nebulization as well as to the mobile phase nature and composition.

In theory,  $b$  is between 0.66 and 2, a high  $b$  value being consistent with low particle size and a low  $b$  value with high particle size.

It has been shown that a  $b$  value can be modified with the solute complexation,<sup>9</sup> instrument settings,<sup>10</sup> nature of nebulization gas<sup>11</sup> or with micro ( $\sim 10$ – $50$   $\mu\text{L}/\text{min}$ ) or capillary ( $\sim 3$ – $5$   $\mu\text{L}/\text{min}$ ) mobile phase flow-rate.<sup>12-16</sup> The  $a$  value has been studied less than the  $b$  value, except for studies on sensitivity in micro or capillary chromatography or with the addition of specific compounds that provide an enhancement of the ELSD response.<sup>16-18</sup>

These two numerical coefficients  $a$  and  $b$  are related to the instrument (gas flow-rate, temperature of evaporation etc.), to the elution conditions (mobile phase nature and flow-rate etc.) and to the concentration and nature of the solute,<sup>16,19</sup> so  $a$  and  $b$  values vary with these parameters.

A reverse variation tendency between these two  $a$  and  $b$  values was early mentioned by Guiochon<sup>20</sup> and evoked today by different authors,<sup>16-18,21-22</sup> which noted a decrease in the  $b$  value at the same time as an increase in the  $a$  value. This can be explained by a possible change of the droplet size distribution during their migration through the drift tube. The aerosol that enters the light scattering cell is not a “primary” aerosol but is rather a “tertiary” aerosol.<sup>23</sup> The scattered light intensity is directly given by the tertiary aerosol, which results from the dependence of the primary aerosol on the nature of the solvent.<sup>20</sup> Particle size plays a major role in determining the efficiency of analyte transport providing an indirect effect on the performance of the detection system.<sup>23</sup>

The goal of this article is to demonstrate by new results and compilation of previous literature data that the two numerical coefficients  $a$  and  $b$  are dependent. This opens an investigation to better describe a quantitative relationship between peak area and injected mass.

### Experimental data

**Chemicals:** Triacylglycerols (tricaprylin CyCyCy, tricaprinc CCC, trilauric LaLaLa, trimyristic MMM, tripalmitic PPP, tristearic SSS, triolein OOO, trilinolein LLL, trilinolenin LnLnLn, PLL, POL, OPO and OSO) were obtained from Sigma-Aldrich (Saint-Quentin Fallavier, France). The ethoxylated alcohols: diethylene glycol monotetradecyl ether ( $C_{14}EO_2$ ),

tetraethylene glycol mono-octadecyl ether ( $C_{18}EO_4$ ), hexaethylene glycol mono-octadecyl ether ( $C_{18}EO_6$ ), octaethylene glycol mono-octadecyl ether ( $C_{18}EO_8$ ) and the esters: palmitic acid myristyl ester ( $C_{16}E_{14}$ ), palmitic acid palmityl ester ( $C_{16}E_{16}$ ), palmitic acid stearyl ester ( $C_{16}E_{18}$ ), palmitic acid arachidyl ester ( $C_{16}E_{20}$ ), palmitic acid behenyl ester ( $C_{16}E_{22}$ ) were purchased from Fluka (Buchs, Switzerland). The parabens (methyl, ethyl, propyl, butyl) and erythromycin were obtained from Fluka and Sigma-Aldrich respectively.

Acetonitrile (Acros, Noisy le Grand, France), acetone and methylene chloride (Carlo Erba, Milan, Italy) were of HPLC grade. HPLC-grade water was obtained by a purelab UHQ PS purification system (Elga, Le Plessis Robinson, France). Trifluoroacetic acid (TFA) was obtained from Carlo Erba. Pentanol, glycerol, nitromethane, nitropropane, DMF and DMSO were purchased from Merck (Darmstadt, Germany); ethanol, isopropanol, propionitrile and ethyl acetate from Prolabo (VWR international, Fontenay-sous-bois, France); trimethylphosphate and polyethylene glycol 4000 from Fluka.

**Equipment:** For the experiments developed in non-aqueous reversed-phase chromatography (NARP), the chromatographic system consisted of a Model LC-6A pump (Shimadzu, Kyoto, Japan), a Model 7125 injection valve with a 10  $\mu$ L loop (Rheodyne, Rohnert Park, California, USA) and a Model Sedex 75 light-scattering detector (Sedere, Alfortville, France), and chromatograms were recorded using an Azur (v 3.0) acquisition software (Datalys, Saint-Martin d'Hères, France).

For the experiments developed in partial aqueous reversed-phase chromatography (PARP), the chromatographic system consisted of a Model 1050 pump (Agilent Technologies, Wilmington, Delaware, USA), a Model 7125 injection valve with a 10  $\mu$ L loop (Rheodyne) and a Model

Sedex 85 low temperature evaporative light-scattering detector (Sedere) with a nebulizer model 04-75 CC, and chromatograms were recorded using an EZ Chrom acquisition software (Scientific Software, California, USA).

For NARP, two sets of data have been studied. The first one comes from a previous publication showing a variation of the *b* value with the modification of the mobile phase nature and composition using post-column addition of different solvents.<sup>24</sup>

In this instance, the nebulizing gas was air at 3.6 bars, the nebulization temperature was set at 37 °C or 55 °C, according to the solvent used post-column, and the gain (PM) was kept at 11.

The column temperature was controlled using an Igloo-cil oven (Cluzeau, Sainte-Foy-la-Grande, France) and set at 20 °C. A Kromasil  $C_{18}$  (5  $\mu$ m), 150  $\times$  4.6 mm column was used (Touzart and Matignon, Les Ulis, France). The mobile phase was MeCN/ $CH_2Cl_2$  65/35 with a flow-rate of 1 mL/min. For the post-column addition, a model 114 M pump in micro mode (Beckman Coulter, Gagny, France) and a pulsation damper (Touzart and Matignon) were used and connected between the column output and the ELSD nebulizer via a polyetheretherketone (PEEK) tee in which the main flow comes from the orthogonal branch of the tee and the added flow from the top branch of the tee. The post-column addition flow-rate was 50  $\mu$ L/min and such a device allows a good mixture of the two flows.

The second set of data in NARP are obtained with new experiments. In this instance, the nebulizing gas was air at

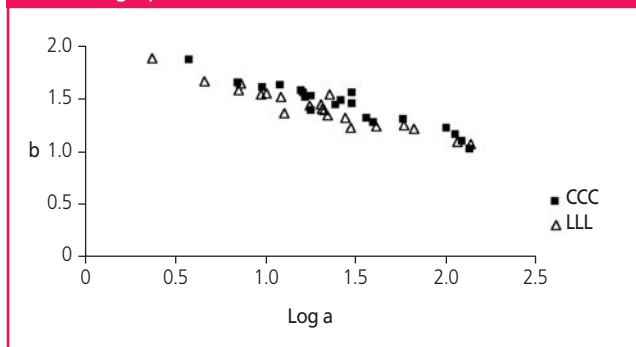
**Table 1:** Equations of the plot *b* vs log *a* obtained for different compounds at different temperatures of the drift tube in NARP (see experimental section for chromatographic conditions).

Solute	Equation
LnLnLn	$b = -0.55 \log a + 1.93 \quad r^2 = 0.9589$
LLL	$b = -0.50 \log a + 1.96 \quad r^2 = 0.9827$
OOO	$b = -0.49 \log a + 1.98 \quad r^2 = 0.9944$
CCC	$b = -0.47 \log a + 2.09 \quad r^2 = 0.9836$
CyCyCy	$b = -0.49 \log a + 2.14 \quad r^2 = 0.9794$
LaLaLa	$b = -0.46 \log a + 2.08 \quad r^2 = 0.9487$
MMM	$b = -0.54 \log a + 2.17 \quad r^2 = 0.9316$
$C_{16}E_{14}$	$b = -0.84 \log a + 2.29 \quad r^2 = 0.9368$
$C_{16}E_{16}$	$b = -0.50 \log a + 2.06 \quad r^2 = 0.9994$
$C_{16}E_{18}$	$b = -0.56 \log a + 2.00 \quad r^2 = 0.9681$
$C_{16}E_{20}$	$b = -0.49 \log a + 1.86 \quad r^2 = 0.9845$
$C_{16}E_{22}$	$b = -0.41 \log a + 1.76 \quad r^2 = 0.9623$
$C_{14}OE_2$	$b = -0.86 \log a + 2.43 \quad r^2 = 0.9997$
$C_{18}OE_6$	$b = -0.63 \log a + 2.22 \quad r^2 = 0.9685$
$C_{18}OE_8$	$b = -0.63 \log a + 2.17 \quad r^2 = 0.9545$

**Table 2:** *b* and log *a* values for two triacylglycerols (CCC and LLL) studied with MeCN/ $CH_2Cl_2$  (65/35) mobile phase and post-column addition of different solvents (see experimental section for chromatographic conditions).

post-column	log <i>a</i> (CCC)	<i>b</i> (CCC)	log <i>a</i> (LLL)	<i>b</i> (LLL)
without	1.21	1.55	0.97	1.54
MeCN	1.2	1.57	1	1.55
MeOH	0.58	1.86	0.37	1.89
EtOH	0.85	1.64	0.66	1.67
iPrOH	1.22	1.51	1.08	1.52
Pentanol	1.39	1.43	1.32	1.39
Glycerol	1.48	1.45	1.24	1.43
MeCN/ $H_2O$ (50/50)	1.6	1.27	1.47	1.22
Nitromethane	2.05	1.15	2.13	1.07
Trimethylphosphate	2.13	1.02	1.61	1.24
[PEG] 0.3 $\mu$ M	2.09	1.09	2.06	1.09
[PEG] 0.16 $\mu$ M	1.76	1.3	1.82	1.21
Acetone	0.98	1.6	0.85	1.58
Ethyl acetate	1.08	1.62	0.86	1.64
DMF	1.48	1.55	1.35	1.54
DMSO	1.25	1.52	1.44	1.32
Nitropropane	1.25	1.38	1.1	1.36
Propionitrile	2	1.21	1.76	1.25

**Figure 1:**  $b$  vs  $\log a$  for two triacylglycerols studied with a given mobile phase (MeCN/CH<sub>2</sub>Cl<sub>2</sub> (65/35)) and post-column addition of different solvents (see experimental section for chromatographic conditions) ■: CCC, △: LLL



3 bars, the drift tube temperature was 40, 50 or 60 °C, and the gain (PM) was kept at 11. The column temperature was controlled using an Igloo-cil oven (Cluzeau), and set at 30 °C. A Kromasil C<sub>18</sub> (5 µm), 250 × 4.6 mm column was used (Touzart and Matignon). The mobile phase flow-rate was 1 mL/min, the gradient MeCN/CH<sub>2</sub>Cl<sub>2</sub> 70/30 to 10/90 in 25 min.

For PARP, the nebulizing gas was air at 3.5 bars, the drift tube temperature was 35 °C, and the gain (PM) was kept at 5. Analysis used a gradient elution at 1 mL/min (A = Water + 0.1% TFA; B = MeCN + 0.1% TFA;  $t = 0$  95% A,  $t = 2.75$  min 95% B) and a Zorbax SB C8 (3.5 µm) 50 × 4.6 mm column (Agilent Technologies).

**Methods:** For studies in NARP coming from a previous publication,<sup>24</sup> the calibration curves were established using between five (range 5–40 ppm) and eight (range 5–100 ppm) concentration levels of two triacylglycerols (CCC and LLL). The difference in the calibration ranges was because of the saturation of the signal with some of the post-column solvent. Each area value was the average of three reproducible injections.

For original results in NARP, the following solute series (triacylglycerols, ethoxylated alcohols and esters) have been studied. The calibration curves were established using five different concentration levels in the range of 5–50 ppm and three injections per concentration level. For each compound, the calibration curve has been performed twice.

For studies in PARP, four concentration levels in the range 250–1000 ppm were used for four parabens and erythromycin.

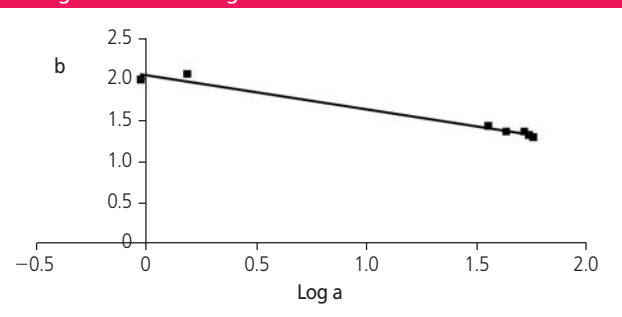
The coefficients  $a$  (or  $\log a$ ) and  $b$  were determined from the logarithmic form of Equation 4.

## Results and Discussion

It is known in ELS detection that the response depends on the nature of the solute. In other respects the mobile phase composition as well as the temperature of the heated drift tube influence the response. So we have chosen different NARP experimental conditions leading to a large range of  $a$  and  $b$  values.

Twenty-two compounds belonging to three different chemical classes (triacylglycerols, ethoxylated alcohols and esters) have been studied with a gradient MeCN/CH<sub>2</sub>Cl<sub>2</sub> at three different temperatures of the heated drift tube (40, 50 and 60 °C). The 22 compounds elute at different retention

**Figure 2:**  $b$  vs  $\log a$  for neomycin in mobile phases with different TFA concentrations. Experimental conditions:<sup>18</sup> standard neomycin solutions in the range 2–50 µg/mL; Waters Spherisorb ODS-2 C<sub>18</sub> (250 × 4.6 mm), isocratic aqueous mobile phase with TFA concentration from 0.3 to 0.9 mL/L, mobile phase flow-rate 1 mL/min, DEDL Sedex 75 (SEDERE) with nitrogen as nebulizer gas.



times to span the gradient profile. For each compound at each temperature of evaporation, the determination of  $a$  and  $b$  values related to Equation 4.  $A = a \cdot m^b$  has been established using linear regression of the plot  $\log A = b \cdot \log m + \log a$ .

**Correlation of  $a$  and  $b$  values with the drift tube temperature variations for a set of different compounds:** As expected, the different compounds eluted at different compositions of the mobile phase and different temperatures of evaporation show different corresponding  $b$  and  $a$  values. In these experiments the  $b$  value varies from 0.99 to 2.6 and  $\log a$  from 0.2 to 1.8.

The highest  $b$  value (2.6) has been obtained for the diethylene glycol monotetradecyl ether analysed with the drift tube temperature at 50 °C (i.e., for one of the most volatile solutes studied with a high drift tube temperature). Such a value is higher than the highest value (2) predicted by the theory.<sup>5</sup> This corresponds to a solute partly evaporated with the mobile phase as previously demonstrated.<sup>25–27</sup> At 60 °C, no signal has been obtained for this compound at 50 ppm, confirming a total evaporation. Such a phenomenon using ELS detection has recently been reported for some pharmaceutical compounds with a nebulizer and an evaporator at high temperatures.<sup>22</sup>

For each compound, and as previously observed,<sup>26</sup>  $b$  and  $\log a$  values change with the drift tube temperature. However, the variation is not random. The equations of the plots  $b$  vs  $\log a$  and the corresponding  $r^2$  values obtained for each studied compound are reported in Table 1. Whatever the compound,  $r^2 > 0.93$  corresponds to a good linear correlation between the two coefficients. Moreover, whatever the compound and its chemical class, the equations are very similar. So, the drift tube temperature influences the  $b$  and  $\log a$  values but these two parameters remain correlated. This correlation depends on the geometry and technology of the ELS detector.

**Correlation of  $a$  and  $b$  values for a given compound with mobile phase composition (NARP and PARP modes) and additive variations:** In a previous publication, we have obtained  $b$  and  $\log a$  values for two triacylglycerols (CCC and LLL) analysed with MeCN/CH<sub>2</sub>Cl<sub>2</sub> (65/35) and post-column addition of different solvents at molar concentrations.<sup>24</sup> These values are reported in Table 2 and the plots  $b$  vs  $\log a$  for each compound are given in Figure 1. A correlation between  $b$  and  $\log a$  values is obtained, the two triacylglycerols being on the same straight

line. Note that such a post column addition allows wide variations in the coefficient values. This gives a highly significant correlation.

Most of the results found in the literature can't be used to determine the correlation between  $a$  and  $b$  because only  $b$  is given. However, we found some data in two recent articles of Megoulas and Koupparis.<sup>18,28</sup>

The first set of data regards the analysis of neomycin in aqueous mobile phases with different TFA concentrations. The authors show that the increase of TFA concentration influences retention time, peak width and asymmetry, and enhances the ELSD response (increase of peak area and coefficient  $a$ ). The plot of the corresponding values ( $b$  vs  $\log a$ ) is given in Figure 2. A relation between  $b$  and  $\log a$  is clearly demonstrated.

These results are in agreement with those of Lane et al.<sup>22</sup> These authors have recently reported linear relationships between  $\log a$  and 5-fluorocytosine elution time as well as  $b$  and 5-fluorocytosine elution time. This non-retained solute was injected at different time intervals through a gradient to establish its three-dimensional response surface as a function of its concentration and the momentary mobile phase composition, expressed as elution time.<sup>22</sup> Indeed, the resolution of the two equations of these two linear relations ( $\log a$  vs elution time and  $b$  vs elution time) leads to a linear correlation between  $b$  and  $\log a$  values.

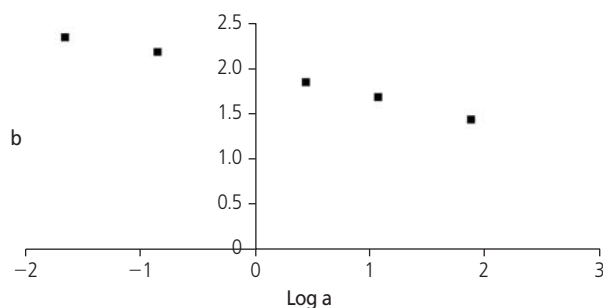
So, these independent experiments show the influence of the composition of the mobile phase (aqueous or not) on the response of a solute. Moreover and above all, these results show a linear correlation between the coefficients  $b$  and  $\log a$ , whatever the detector model and the manufacturer.

**Correlation of  $a$  and  $b$  values for a set of different compounds analysed in an isocratic mobile phase:** In a second article of Megoulas and Koupparis,<sup>28</sup> the set of data concerns the analysis of kanamycin and four gentamicin components in a given aqueous mobile phase. Once again, a significant correlation between  $b$  and  $\log a$  is obtained (Figure 3).

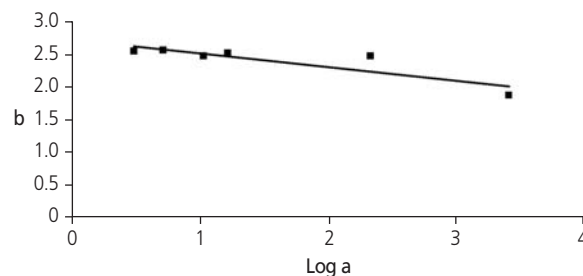
A linear correlation between  $b$  and  $\log a$  values have also been observed for six carbohydrates studied in MeCN/H<sub>2</sub>O (80/20) or in pure water. (from reference 7, results not shown).

These two independent experiments allow us to conclude that different solutes analysed in an isocratic elution lead to different  $b$  and  $\log a$  values but with a systematic correlation between them.

**Figure 4:**  $b$  vs  $\log a$  for parabens (methyl, ethyl, propyl, butyl) and erythromycin studied in aqueous mobile phase (see experimental section for chromatographic conditions).



**Figure 3:**  $b$  vs  $\log a$  for kanamycin and four gentamicin (sulphate) components. Experimental conditions:<sup>28</sup> standard kanamycin solutions in the range 4.5–75  $\mu\text{g/mL}$ , standard gentamicin solutions in the range 35–240  $\mu\text{g/mL}$  of total gentamicin; Waters Spherisorb ODS-2 C18 (250  $\times$  4.6 mm), mobile phase: H<sub>2</sub>O (containing 35.4  $\mu\text{g/mL}$  of trichloroacetic acid and 0.89  $\mu\text{L/mL}$  of TFA)-MeOH-MeCN (990:5:5 v/v/v), flow-rate 1.1 mL/min, DEDL Sedex 75 (SEDERE) with nitrogen as nebulizer gas.



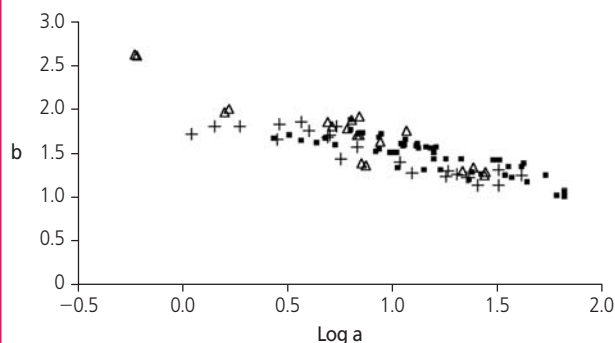
**Correlation of  $a$  and  $b$  values for a set of different compounds using a gradient elution:** Four parabens and erythromycin have been studied with a gradient MeCN/H<sub>2</sub>O in reversed-phase chromatography, showing a correlation between  $b$  and  $\log a$  (Figure 4) using 250, 400, 800 and 1000 ppm concentrations.

Last but not least, in NARP chromatography using a mobile phase constituted by a mixture of organic solvents (MeCN/CH<sub>2</sub>Cl<sub>2</sub>) and a gradient elution, the plot of  $b$  vs  $\log a$  for the 22 compounds belonging to three different chemical classes at three different temperatures of the heated drift tube is reported in Figure 5.

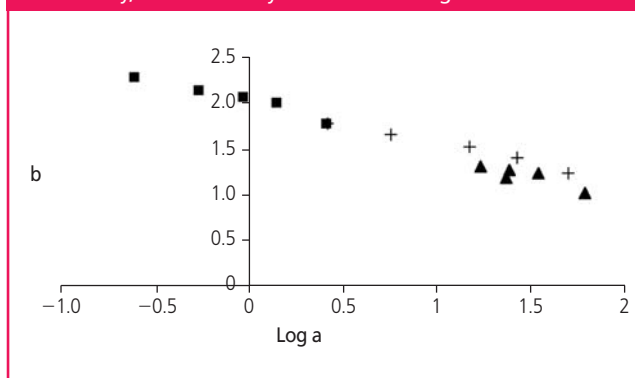
For each class (triacylglycerols, ethoxylated alcohols and esters), a correlation between  $b$  and  $\log a$  is observed as the different solutes of a same class line up (■,  $\Delta$  and + on Figure 5). Moreover, the three classes are on the same straight line showing a generalization of the phenomenon, (i.e., the same correlation between  $b$  and  $\log a$ , whatever the nature of the solute).

Taking account of the three classes of compounds, the equation of the linear regression is  $b = -0.57 \cdot \log a + 2.1$ . If we consider the previous study of the two triacylglycerols in

**Figure 5:**  $b$  vs  $\log a$  for different compounds studied at different temperatures of the drift tube (see experimental section for chromatographic conditions) ■: triacylglycerols,  $\Delta$ : ethoxylated alcohols, +: esters



**Figure 6:**  $b$  vs  $\log a$  for different triacylglycerols studied with a gradient MeCN/CH<sub>2</sub>Cl<sub>2</sub>. Temperature of the drift tube = 40 °C  
 +: first day, ■: second day ▲: after cleaning of the needle.



MeCN/CH<sub>2</sub>Cl<sub>2</sub> with post-column addition of various solvents (data corresponding to Figure 1),<sup>27</sup> the equation is  $b = -0.44 \cdot \log a + 2.02$ . These two equations are very similar showing that the overall data fit on the same straight line ( $b = -0.51 \cdot \log a + 2.06$ ).

As established by Guiochon,<sup>20</sup> the results can be explained by assuming a strong dependence of the phenomena leading to a change of the droplet size distribution with the nature of the solvent. These two sets of data have been obtained with one ELS detector. Therefore, we may conclude that only one correlation between the coefficients  $a$  and  $b$  when the composition of the mobile phase and/or the nature of the solutes and/or the temperature of the drift tube is modified using constant characteristics of the ELSD (i.e., one nebulizer type with one instrument model).

The following experiments show that this correlation is still valid whatever the variation in the instrument. For example, it has been verified with a nebulizer working with a partially plugged needle. Indeed, the analysis of triacylglycerols performed during two full days (reproducibility studies) have given different values of  $b$  and  $\log a$  for one compound, however, the correlation was still identical. After cleaning the needle of the nebulizer, lower values have been obtained, but the correlation remained the same (Figure 6).

## Conclusion

Many parameters influence the response in ELSD. The different studies described above confirm that for a given instrument, the nature of the solute, the composition of the mobile phase, and the temperature of the drift tube affect  $b$  and  $\log a$  values leading to non-linear response curves and variations in sensitivities. Such detector behaviour explains the different studies to improve response as well as quantification in ELSD.<sup>18,28</sup> In particular, two recent articles describe a convenient calibration method to account for these effects to improve the accuracy of quantitative measurements using gradient HPLC–ELSD.<sup>22,29</sup> Another way to facilitate the quantitative analysis could be deduced from our study. Indeed, the goal of this article is to show that when  $b$  changes,  $\log a$  also changes proportionally. These two coefficients are not independent, leading to the complexity of the ELS response based on the diffusion by particles for which we do not control size distribution as well as particle number. Experimental data coming from our lab — for triacylglycerols, ethoxylated

alcohols, esters, parabens, sugars and antibiotics — as well as independent sets of data obtained from different laboratories (neomycin, kanamycin, gentamicin and 5-fluorocytosine) demonstrate the generalization of the dependence of the  $b$  and  $a$  coefficient whatever the ELSD model and technology.

An important consequence of these results is to suppress the problem given by variations of the ELSD response at any day (lack of reproducibility) for quantitative purpose. Slight changes in  $b$  may be compensated by an  $a$  value variation and the commonly used Equation 4, which is a function of two dependent numerical coefficients  $a$  and  $b$  that becomes a function with only one coefficient  $b$ . Development for a new way in ELSD quantitative analysis is in progress in our laboratory.

## References

- G. Guiochon and C.L. Guillemin (Ed.), Quantitative gas chromatography for laboratory analyses and on-line process control. *Journal of Chromatography Library*, vol. 42, Elsevier, Amsterdam, p. 393 (1988).
- R.P.W. Scott in E. Katz (Ed.), Quantitative analysis using chromatographic techniques. separation science series, John Wiley & Sons, p. 1 (1987).
- I.A. Fowliss and R.P.W. Scott, *J. Chromatogr.*, **11**, 1 (1963).
- R.P.W. Scott in E. Katz (Ed.), Quantitative analysis using chromatographic techniques. separation science series, John Wiley & Sons, p. 63 (1987).
- T.H. Mourey and L.E. Oppenheimer, *Anal. Chem.*, **56**, 2427 (1984).
- M. Dreux and M. Lafosse, in Z. El Rassi (Ed.), Carbohydrate Analysis. *Journal of Chromatography Library*, vol. 58, Elsevier, Amsterdam, p. 515 (1995).
- M. Lafosse and B. Herbreteau in Z. El Rassi (Ed.), Carbohydrate analysis by modern chromatography and electrophoresis. *Journal of Chromatography Library*, vol. 66, Elsevier, Amsterdam, p. 1101 (2002).
- A. Stolyhwo, M. Martin and G. Guiochon, *J. Liq. Chrom.*, **10**, 1237 (1987).
- S. Héron, M. Dreux and A. Tchaplá, *J. Chromatogr. A*, **1035**, 221 (2004).
- H. Gangloff et al., French Patent no. 01 09 202 (07-11-2001).
- Y. Mengerik, H.J.C. De Man and S.J. S.Van Der Wal, *J. Chromatogr.*, **552**, 593 (1991).
- M.B.O. Andersson and L.G. Blomberg, *J. Microcol. Sep.*, **10**, 249 (1998).
- R. Trones et al., *J. Chromatogr. A*, **814**, 55 (1998).
- J.N. Alexander IV, *J. Microcol. Sep.*, **10**, 491 (1998).
- S. Héron and A. Tchaplá, *J. Chromatogr. A*, **848**, 95 (1999).
- K. Gaudin, A. Baillet and P. Chaminade, *J. Chromatogr. A*, **1051**, 43 (2004).
- F.S. Deschamps, P. Chaminade and A. Baillet, *The Analyst*, **127**, 35 (2002).
- N.C. Megoulas and M.A. Koupparis, *J. Chromatogr. A*, **1057**, 125 (2004).
- J.M. Charlesworth, *Anal. Chem.*, **50**, 1414 (1978).
- M. Righiezza and G. Guiochon, *J. Liq. Chrom.*, **11**, 1967 (1988).
- S. Lane et al., 25th International Symposium on Chromatography, October 4–8 2004, Paris, France, Poster.
- S. Lane et al., *Anal. Chem.*, **77**, 4354 (2005).
- J.A. Koropechak et al., *Advances in chromatography*, **40**, 275 (2000).
- S. Héron, M. Dreux and A. Tchaplá, LC•GC accepted.
- G.K. Webster, J.S. Jensen and A.R. Diaz, *J. Chromatogr. Science*, **42**, 484 (2004).
- N. Pointereau et al., 25th International Symposium on Chromatography, 4–8 October 2004, Paris, France, Poster.
- A.Stolyhwo, H. Colin and G. Guiochon, *J. Chromatogr.*, **265**, 1 (1983).
- N.C. Megoulas and M.A. Koupparis, *J. Pharm. Biomed. Anal.*, **36**, 73 (2004).
- B.T. Mathews et al., *Chromatographia*, **60**, 625 (2004).

**Sylvie Héron** is assistant professor of analytical chemistry in the Group of Analytical Chemistry of Paris Sud (LETIAM) at the University of Paris Sud (IUT Orsay). Her current research interests include development and optimization of LC methods for lipids analysis with ELSD and MS.

**Marie-Geneviève Maloumbi** is assistant professor in nutrition at the University M. Nguabi, Brazzaville, Congo. She completed her PhD in LETIAM in July 2006 where she developed a new method of quantitative analysis of oils with ELSD.

**Michel Dreux** was a professor in separation sciences at the Institute of Organic and Analytical Chemistry of the University of Orleans, France. He was at the head of the Analytical Research Group. His main research interests concern chromatography, capillary electrophoresis and capillary electrochromatography. He is now the scientific director at SEDERE.

**Eric Verette** got his PhD in Analytical Chemistry at the Faculty of Pharmacy of Montpellier, France. He worked several years at Gilson SA as the evaluation laboratory manager and as the European scientific laboratory manager. He is now the marketing manager at SEDERE.

**Alain Tchaplal** is professor of analytical chemistry in the Group of Analytical Chemistry of Paris Sud (LETIAM) at the University of Paris Sud (IUT Orsay). He is head of the LETIAM research group. His main research interests concern chromatography, mass spectrometry and liquid stationary phases characterization.

AI  
COM

