Gas Chromatography with UV-Vis Molecular Absorption Spectrometry Detection: Data Acquisition and Treatment when Using a Diode-Array Spectrophotometer

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Key Words

Gas chromatography
UV-Vis detection
Gas phase absorption spectrometry
Alcohols

Summary

This article describes a theoretical/practical study of the parameters which affect the chromatograms obtained when using a molecular diode-array spectrometer as the detector in gas chromatography. The objective is to offer some rules which permit the identification of the optimum signal/noise relation. To achieve this, we study the effect of the different parameters which affect the noise and how to reduce their impact, as well as alternatives for increasing the signal. All the options tested can be applied by correctly programming the spectrometer with BASIC programs, within reach of any user who has even a small understanding of programming. Finally, we consider the effect that the selection of the acquisition conditions may have on the chromatographic resolution. All the studies are carried out using a mixture of alcohols and phenols.

Introduction

Theoretical Background

The objective of the study was to optimize the working conditions of the detector to achieve the maximum possible signal/noise (S/N) ratio; that is, to obtain the lowest possible noise and the highest possible signal.

Noise Minimization Methods

During data acquisition. It is well known that experimental noise can have two origins, chemical or instrumental. In our detection system, the possibility of chemical noise can be discounted, since there are no reactions or other chemical processes taking place during the detection. The instrumental noise also has two sources: that associated with the electronic components, and that relating to the optical components.

Instrumental noise associated with electronic components. The most significant of these [1] are the Johnson noise (N_t), the shot noise (N_d), the flicker noise (N_p) and the environmental noise (N_a).

Instrumental noise associated with optical components. There are two elements which can be considered as important: noise related to fluctuations in the position of the spectrometer cell, which has similar behaviour to N_d, and the noise associated with working at very low signals, which is similar to N_t.

The instrumental variables which can be modified during a chromatogram, and which can affect the types of noise mentioned above, are temperature, time constant (t) and wavelength.

- The temperature only affects N_t; its impact on the noise increases with its square root.
- The detector's time constant, t, gives an idea of the detector's response time. In principle, this time is unknown and is constant for each detector. However, the functioning of multichannel detectors (such as Fourier-transform or diode-array units) can mean that this parameter may be similar to the integration time, t_i [1]. The integration time affects the various types of noise in different ways:

$$N_t \text{ and } N_d \text{ are proportional to } (t_i)^{-1/2}$$
$$N_p \text{ and } N_a \text{ are proportional to } t_i \qquad (1)$$

This means that N_p and N_a decrease when using low integration times, while N_t and N_d decrease with high integration times, although not so drastically.

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The wavelength has an effect since the amount of light circulating in the instrument depends on it. At the lowest wavelengths (around 190–196 nm, the instrument's limit) the quantity of light involved is lower because the optical components, despite being quartz, absorb some of these radiations; a higher noise level is therefore observed for these wavelengths.

During the process. A method for reducing the noise of a signal which has already been recorded is the use of algorithms or mathematical procedures which make it possible to extract the signal from the background noise. Most of these methods are based on the fact that when a measurement is taken repeatedly, the signal does not vary but the noise does, falling by a factor of approximately $n^{1/2}$ where n is the number of repetitions. There are different methods which can be applied, such as Boxcar Averaging, Smoothing, Ensemble Averaging and Digital Filtering [2, 3].

Ways to Increase the Signal

This is only possible *while acquiring the data.* The molar absorptivity of a compound is an intrinsic property which cannot be altered, although it is possible to alter the compound's $\varepsilon_{apparent}$ molar absorptivity. There are three ways to do this:

- Adjust the conditions of the media (solvent, ionic force or refractive index) which is not possible due to working in the gas phase;
- Modify the instrument's spectral slit width, not possible with a diode-array spectrophotometer;
- Use the sum of all the compound's absorbance wavelengths as the measurement parameter. This alternative can be tested.

To investigate the above possibilities, a mixture of alcohols and phenols was chosen; this group of compounds offered a moderate sensitivity with this technique. The compounds chosen were methanol, ethanol, 2-propanol, 1-butanol, 1-pentanol, 1-hexanol, phenol, 2-nitrophenol, 2,3-dimethylphenol, 2,4-dimethylphenol and benzyl alcohol.

Experimental

Apparatus

All measurements were performed by using a Hewlett-Packard model HP 8451A diode-array spectrophotometer furnished with a quartz flow cell of 1-cm path length (Hellma 174 QS) and equipped with a Keyboard (HP98155A), a floppy disk drive for bulk data storage (HP9121) and a graphics plotter (HP7475A).

A modified Hewlett Packard 5890 series II gas chromatograph was used, equipped with a 4 m × $1/8^{in}$ packed column filled with 5 % SE-30 on Chromosorb W HP 80/100. The FID detector was eliminated and 20 cm of the chromatographic column was taken outside the chromatograph through the FID hole. A home-made temperature controller was used for heating the column outside the oven in order to maintain the chromatographic resolution.

A HP 89090A Peltier temperature control accessory and a home-made temperature controller were employed for flow cell heating up to 70 °C and 400 °C, respectively.

Reagents and Solutions

All chemicals used were analytical reagent grade. Petroleum ether (Carlo Erba, HPLC quality) was used as the solvent. The test compounds used were: ethanol 99.8 %, 2-propanol 99.8 %, 1-butanol 99.8 %, 1-pentanol 99 %, 1-hexanol 99 % and phenol 99.5 % from Carlo Erba, and methanol 99.9 %, 2-nitrophenol 99 %, 2,4-dimethylphenol 98 %, 2,3-dimethylphenol 99 % and benzyl alcohol 99 % from Aldrich.

Stock solutions of the liquid test-substances (methanol, ethanol, 2-propanol, 1-butanol, 1-pentanol, 1-hexanol, benzyl alcohol and 2,4-dimethylphenol) were prepared by diluting 20 µL aliquots in 5 mL of petroleum ether. Stock solutions of phenol, 2,3-dimethylphenol and 2-nitrophenol were prepared by dissolving the appropiate amounts of the compounds in petroleum ether. Working standard solutions were prepared daily by serial dilution of the stock solution.

Description of the System. Procedure

The Gas Chromatography-Gas Phase Molecular Absorption Spectrometry system is simple [4]. The FID of the chromatograph was eliminated and part of the packed column was taken outside and connected directly to the spectrophotometer flow cell (which is heated by the Peltier at 70 °C or by a home-made heater to higher temperature). In order to keep the oven and the outside column at the same temperature, a simple heating and temperature control system was built. Two meters of high-temperature, heater hook-up wire, insulated with silicone rubber, was wound around the outside of the column and connected to a variable transformer.

The noise was calculated as the standard deviation of the background noise over 15 seconds; it can also be calculated as 1/5 of the difference between the highest and lowest background noise level over the same period.

The required BASIC programmes were created for each of the different options discussed in this paper.

Results and Discussion

The different procedures used to improve the S/N ratio in the determination of the test substances are described below.

Table I. Noise at different cell temperatures and wavelengths.

Temperature	190 nm	200 nm	220 nm	238 nm	250 nm	300 nm
150 °C	0.001105	0.000454	0.000304	0.000373	0.000354	0.000427
200 °C	0.001226	0.000603	0.000294	0.000314	0.000378	0.000463
220 °C	0.001104	0.000674	0.000280	0.000376	0.000355	0.000417
250 °C	0.001105	0.000722	0.000337	0.000328	0.000383	0.000417
280 °C	0.000943	0.000753	0.000281	0.000402	0.000384	0.000493
300 °C	0.001066	0.000639	0.000331	0.000384	0.000369	0.000389

Table II. Improvement in the Signal/Noise ratio using the maximum S/N wavelength instead of the maximum absorption wavelength.

Compound	Maximum absorbance wavelength (nm)	Maximum S/N wavelength (nm)	S/N Improvement
Methanol	190	198	1.06
Ethanol	190	198	1.04
2-Propanol	190	198	1.09
1-Butanol	190	198	1.05
1-Pentanol	190	198	1.06
1-Hexanol	190	198	1.06
Phenol	190	214	2.90
Benzyl alcohol	190	212	1.60
2.4-Dimethylphenol	190	190	1.00
2.3-Dimethylphenol	190	190	1.00
2-Nitrophenol	208	260	2.60

Improvement During Data Acquisition: Minimizing the Noise

As explained above, there are three parameters which can be controlled: the temperature of the detector and of the electronic components, the integration time and the measurement wavelength.

Influence of the Temperature

The temperature of the electronic components could only be altered by installing thermostatic systems in the unit's interior. However, the cell itself is a focus of heat in the instrument which could have some effect on the signal; tests were therefore carried out by heating the cell to different temperatures and calculating the noise at various wavelengths. The results are shown in Table I. Using these data (which were normalized in order to remove their dependence on the wavelength), a variance analysis was made and it was found that the values obtained at the various temperatures were not significantly different, confirming that the noise is not affected by the temperature.

Influence of the Integration Time on the Noise

In a previous investigation, we carried out a detailed study on the effect of the integration time on the noise at different wavelengths (see Figure 4 in reference [4]). The results of that study indicated that the optimum integration time, for the wavelengths studied, was 0.5 s. The results agree well with expression (1) above.

Correct Selection of Wavelengths

From the results above, it is possible to obtain what we call the noise spectrum, which indicates its variation with wavelength (see Figure 5 in reference [4]). Precisely because the noise varies with wavelength, the choice of a wavelength of maximum absorption as the optimal one can give rise to errors, since the noise at that wavelength should be taken into account. When selecting the best measurement wavelength for each compound, it is therefore best to calculate each signal/noise spectrum; this is done by dividing the absorption spectrum by that of the noise. The S/N spectra for the compounds are shown in Figure 1; Table II gives the wavelengths of the greatest S/N ratio for each compound, together with the maximum absorption wavelength and the increase in the S/N ratio obtained when working with the former compared to the latter. This relation indicates exactly the amount of improvement obtained on the detection limit when working with these wavelengths.

Improvements During the Acquisition of the Data: Signal Increase

Since the photodiode spectrometer can make complete scans in a very short space of time, it is possible to use the sum of all, or some, of each compound's absorption

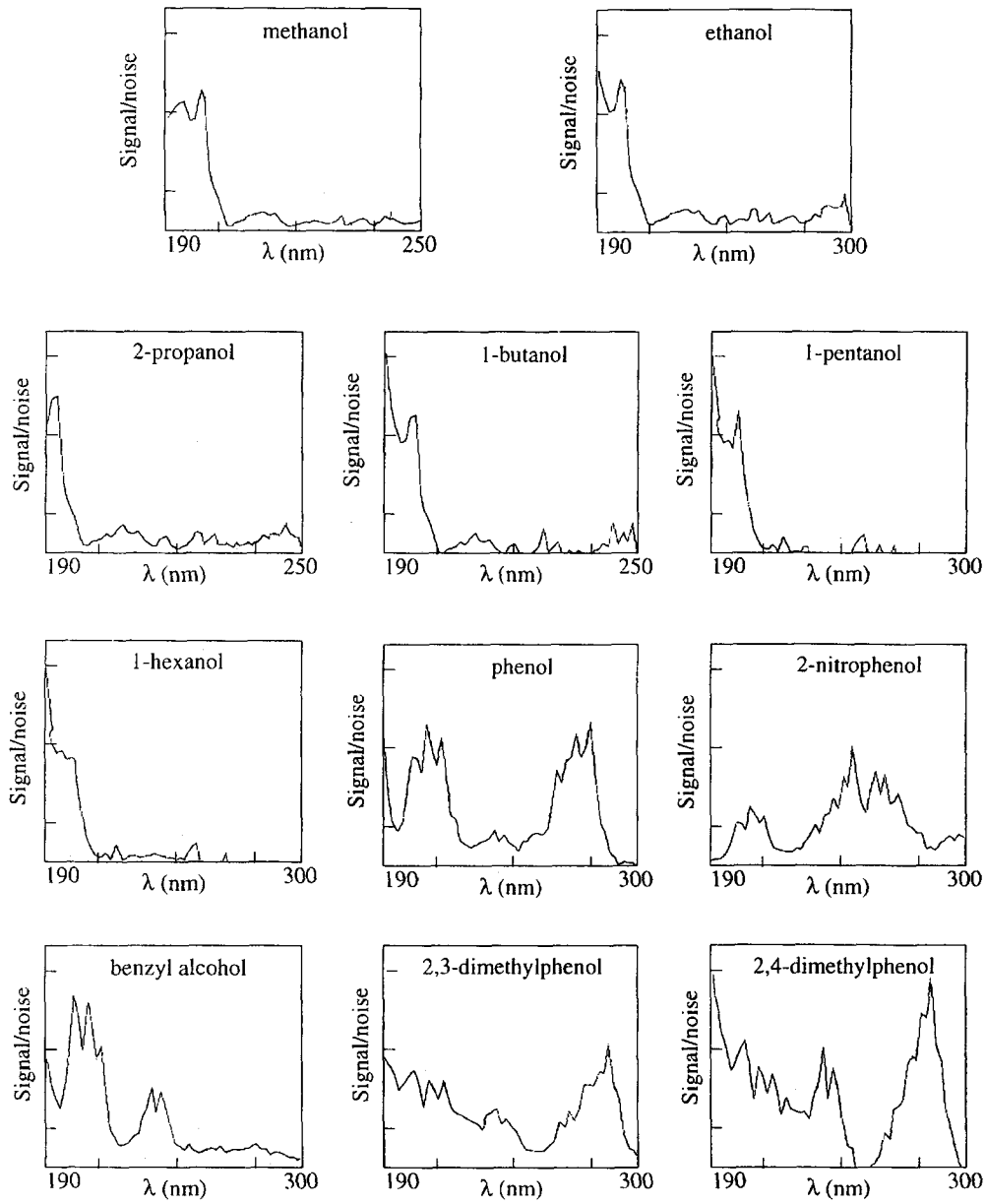

Figure 1
Signal/Noise spectra of the test compounds.

wavelengths as a parameter. However, totalling up the wavelengths also means adding together the noise elements, making it very important to consider only such intervals as will contribute more to the signal than to the noise.

The study of a compound's optimum range starts from its S/N spectrum. The maximum is located and wavelength ranges are sought whose S/N value is 90, 80, 70, 60, 50, 40, 30, 20 or 10 % of this maximum; the new S/N ratio is then calculated for each of these ranges. This present study investigated two substances, methanol and phenol, separately; they were chosen because they represent the two families, aliphatic and aromatic, which are worked with. The results for methanol are given in Table III and those for phenol in Table IV, indicating in each the increase in the S/N ratio obtained for each wavelength range against that obtained for the maximum.

In the case of methanol, the improvement in the S/N relation was slight, being no more than 1.5 times. This is due to the fact that the wavelengths which were added up correspond to zones with quite a lot of noise. In the case of the phenol, the increase was greater and reached nearly 4 times with respect to the maximum value. Having seen these results, it can be concluded that this procedure can indeed achieve moderate improvements. There are two negative aspects, however:

- The selection of the optimum wavelength range cannot be generalized;
- The use of this alternative involves losing one of the detector's advantages, which is the selectivity available from the selection of the wavelength.

Table III. Improvement in the Signal/Noise ratio for methanol using different wavelength ranges.

Wavelength (nm)	Improvement S/N	%
190	1.0	
198	1.1	
192–198	1.1	80
190–198	1.2	70
190–200	1.3	50
190–202	1.3	40
190–204	1.4	10
190–210	1.5	0

%: the wavelength range is whose the signal/noise value is the % of the signal/noise maximum.

Improvement by Treatment of the Data: Noise Reduction

Of the different methods which can be applied (Boxcar Averaging, Smoothing, Ensemble Averaging and Digital Filtering), and given the characteristics of the available instrumentation, the best option is probably Smoothing [2, 3].

To do this, the analytical signals obtained are divided into zones or groups, known as windows. The points within each window are averaged and the resulting value is substituted for all the points in that window. This window is mobile, moving point by point along the whole signal in such a way that each point of the original signal appears in several windows and, therefore, in several averagings.

In principle, averages can be taken from the two dimensions obtained during the chromatogram, time and wavelength. The wavelength average uses as the signal the average of the absorbance at all or some of the compound's absorption wavelengths; however, this option is mathematically similar to that of the sum of absorbances as described above. The time average involves running the chromatogram to average the signal values obtained over different time intervals. It might be thought that this averaging would be equivalent to the integration time. However, due mainly to the flick noise as seen above, the use of integration times greater than 0.5 s does not improve the S/N ratio, while averaging the relation gives a greater improvement with greater averaged time interval.

The effect of the time averaging was investigated for the two compounds (methanol and phenol) and the results are given in Table V. To aid comprehension of the effect, two possibilities were considered: working at the wavelength of best S/N ratio (198, 214 nm respectively), and working at the absorbance sum which offered the best result from the last section (190–210 for methanol and 202–220, 266–278 for phenol).

The following conclusions can be drawn from the results obtained.

Table IV. Improvement in the Signal/Noise ratio for phenol using different wavelength ranges.

Wavelength (nm)	Improvement S/N	%
190	1.0	
214	2.0	
212–216, 268–270	2.0	90
210–216, 268–270	2.4	80
208–216, 268–270	2.8	70
206–218, 266–276	2.5	50
202–220, 266–278	3.6	40
198–220, 262–278	3.2	30
190–222, 256–280	2.5	20
190–228, 248–282	2.1	10
190–290	2.4	0

%: the wavelength range is whose the signal/noise value is the % of the signal/noise maximum.

Table V. Effect of the time averaging on the Signal/Noise ratio.

	Methanol		Phenol	
Window	S/N at 198 nm	S/N at 190–210 nm	S/N at 214 nm	S/N at 202–220, 266–278 nm
0	61	86.84	684	1238.15
3	90	117.44	1129	1832.74
5	146	121.13	1839	2299.20
7	174	122.59	2154	2251.87
9	218	90.74	2204	2637.31

- An increase in the window's size generally improves the S/N ratio, although this increase is not proportional to the square root of the window size.
- For methanol, the best results were obtained by using the wavelength of maximum S/N ratio and an averaging of 9. With the 5 and 7 averaging, an improvement in the S/N ratio of 2.5 times was obtained with respect to the same, un-averaged wavelength; the improvement was 3 times with respect to the 190 nm wavelength.
- For phenol, similar results were obtained using the wavelength of maximum sensitivity. There were increases in the S/N ratio of 3 times with respect to the same, un-averaged wavelength, and between 6 times with respect to the un-averaged maximum absorption wavelength. The improvement obtained when the two alternatives (wavelength selection and smoothing) were combined can be appreciated. On using wavelength ranges, slightly higher increases were obtained, of between 1 and 1.4 times better that that obtained from the maximum S/N ratio wavelength; this improvement does not compensate for the loss of selectivity involved. The optimum window size was 9, although good results were also obtained with values of 5 and 7.

Table VI. Resolution obtained using different window sizes.

Mixtures	Window size 0 points	Window size 3 points	Window size 5 points	Window size 7 points	Window size 9 points
Methanol + 1-Butanol	3.08	2.65	2.10	1.63	1.28
Methanol + 2-Propanol	1.40	1.25	0.94	0.76	0.71
Methanol + Ethanol	1.06	0.94	0.77	0.59	0.50

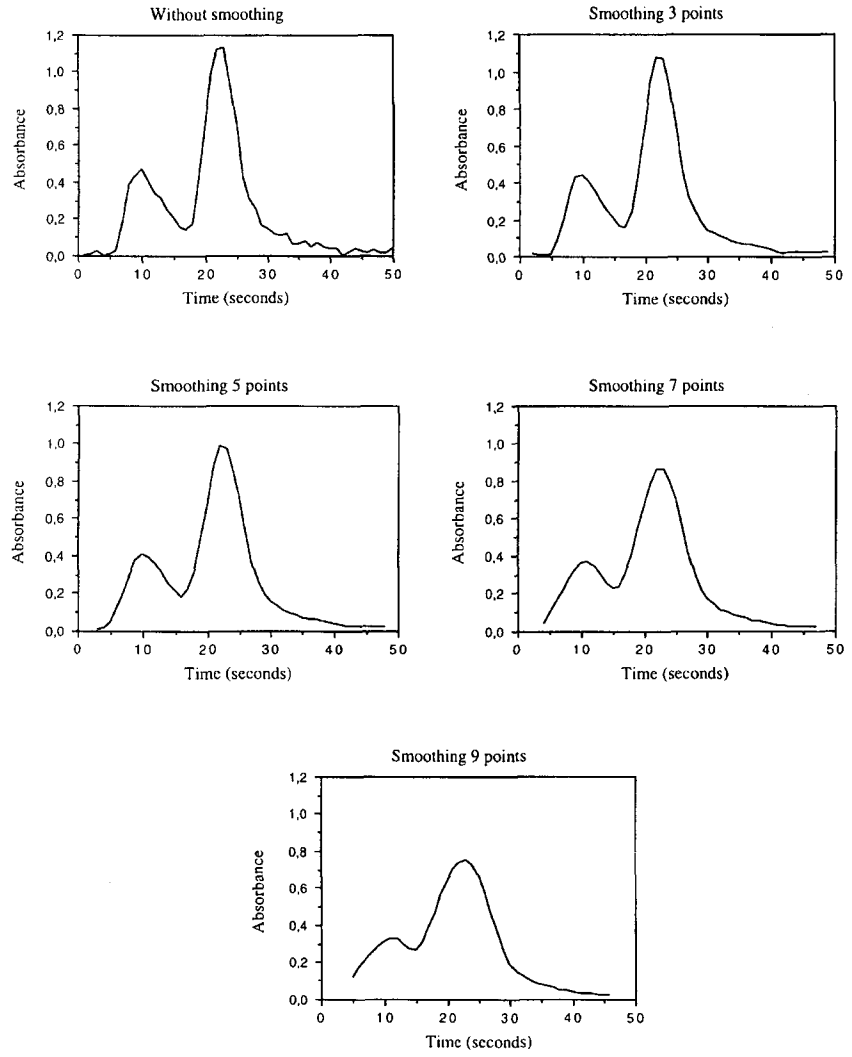

Figure 2
Chromatograms obtained for the methanol/2-propanol mixture at the different smoothing values.

The Effect of Time Averaging on Chromatographic Resolution

The time-smoothing carried out has the disadvantage of negatively influencing the chromatographic resolution. On averaging, the chromatographic peaks lose height and become wider, so that the resolution is lower.

An experimental study was carried out to investigate the effect of averaging on the resolution of three alcohol mixtures with a different initial resolution. These were methanol/1-butanol, methanol/2-propanol and methanol/ethanol. These mixtures were chosen because the temperature programme used meant that they would respond to three different chromatographic situations.

Chromatograms were produced for the three pairs, at the same wavelength and using different windows (0, 3, 5, 7, 9) and the resolution for each pair was calculated at each window; the results are given in Table VI. By way of example, Figure 2 shows the chromatograms obtained for methanol/2-propanol pair at the different smoothing values.

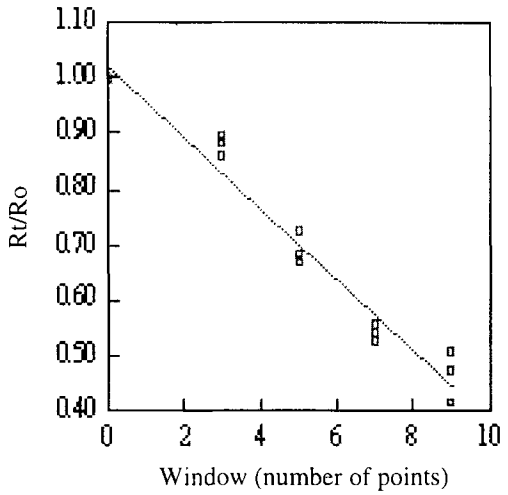

Figure 3
Variation in R_t/R_o against the window size, t_v. (R_t = resolution obtained for a particular window size and R_o = resolution obtained without smoothing).

A very interesting result can be obtained from Table VI, as indicated in Figure 3. This figure shows the variation in R_t/R_o against the window size, t_v, where R_t is the resolution obtained for a particular window size and R_o is that obtained without smoothing. As can be seen, the results obtained for the alcohol pairs vary according to a linear relation ship common to all of them. The equation is:

$$R_t / R_o = 1.02 - 0.0635\, t_v$$

Using this equation, we can predict the resolution which will be obtained for two compounds in terms of t_v, when the un-averaged resolution is known. Given that increased t_v gives a better S/N ratio, the best t_v for any pair of compounds can be predicted, permitting an optimum S/N ratio for a given resolution. The results also indicate that this expression is generalizable to any other compound pair.

Conclusions

The following general conclusions can be drawn from all our studies carried out.

- From the point of view of data acquisition, the best integration time (which gives the lowest noise) is 0.5 s.
- The cell temperature does not influence the noise; work can therefore be undertaken at the temperature necessary for each case.
- As regards the attempts to improve the signal/noise ratio, once all the data were analyzed, it is considered that the best option is to work with the wavelength of maximum S/N ratio for each compound and then apply a smoothing of 5 points. In this way, the loss of chromatographic resolution is small and resolution is not affected by the wavelength. In some specific cases, the window size can be increased even further.

Table VII. Comparison of detection limits*.

Compound	D.L.[a]	D.L.[b]	$(DL)^b/(DL)^a$
Methanol	74	27	2.7
Ethanol	47	42	1.1
2-Propanol	55	29	1.9
1-Butanol	120	43	2.8
1-Pentanol	66	36	1.8
1-Hexanol	69	34	2.0
Phenol	4	0.8	5.0
Benzyl alcohol	15	1.1	13.6
2-Nitrophenol	2.5	0.3	8.3
2.4-Dimethylphenol	10	1.9	5.3
2.3-Dimethylphenol	10	2.3	4.3

*Concentration in $mg \cdot L^{-1}$
D.L.: Detection Limit
[a] Obtained working at the maximum absorbance wavelength
[b] Obtained working at the maximum S/N wavelength and 5 points smoothing

In order to better compare the results obtained, a joint calibration study was carried out for the different test substances, working at the maximum absorbance wavelength and in the conditions described above as optimum. The detection limits (DL) obtained were:

$$DL = 3s/m$$

where s is the base line standard deviation for the chromatogram and m is the slope of the calibration curve. The results are given in Table VII, indicating the improvements which can be obtained from the use of data treatment. As can be seen in the case of the aromatic compounds the improvement in DL is more than an order of magnitude; it will therefore be useful to continue applying this type of treatment to future determinations.

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