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Improved chromatographic analysis of volatile sulfur compounds by the static headspace technique on water–alcohol solutions and brandies with chemiluminescence detection

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Abstract

Volatile sulfur compounds are renowned for their nauseous character in alcoholic beverages at an extremely low concentration. In this work, a quantitative analytical method was developed for their detection with the static headspace technique using a Sievers chemiluminescence detector (SCD 355). It gives a linear response over the concentration range 10–100 $\mu\text{g/l}$ with a repeatability or reproducibility error of less than 5%. It was found that the headspace concentration of sulfur compounds in water–alcohol solutions or brandies rises with increasing ratio between the gas and liquid phase volumes and is proportional to the temperature. However, it diminishes with increasing ethanol content and is barely sensitive to the liquid-phase salt concentration.

1. Introduction

Nauseous volatile sulfur products such as hydrogen sulfide (H_2S), carbonyl sulfide (COS), sulfur dioxide (SO_2), thiols (RSH), sulfides (RSR), polysulfides (RS_nR , $n = 2, 3, \dots$) or thioesters (RCOSR') are found in grape juices [1], wines [2,3], beers [4–6] and spirits [7,8]. Most of these compounds are the cause of olfactory problems and in some cases organoleptic defects, because of their nauseous nature and their very low perception levels [9–11]. However, some of them (with medium and high boiling points) may have a positive influence [12,13].

Sulfur products have diverse origins: (1) natural, from plant cell breakdown products; (2) plant protection via breakdown of vine plant treatment products in wines [14,15]; (3) fermentation [16–19]; (4) thermal, via Maillard and Strecker reactions during distillation; and (5) photochemistry, in the case of white wines [20,21].

The determination of these compounds in wines and spirits is difficult because of their volatility and their very low olfactory perception levels, which require the use of highly sensitive detectors. Among the different analytical techniques, gas chromatographic separation coupled with sulfur-specific detection, namely flame photometric detection (FPD), is the most widely used [22,23]. The detection principle is based on

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the burning of sulfur products to place the sulfur atom in an excited state (S_2^*), which emits light that can be detected at 393 nm with a photomultiplier (PMT) [24].

More recently, a new type detector has been developed. This chemiluminescence detector (SCD type) works through the burning of sulfur products to yield sulfur monoxide (SO). A highly pure ceramic is subsequently used to collect 90% of the related combustion products, which are then channelled under vacuum towards the chemiluminescence detector [25,26]. In the reaction cell, SO reacts with ozone to yield a sulfur oxide molecule in the excited state. This latter compound emits light while returning to its ground state, and this light signal can be detected with a PMT (Fig. 1).

This type of detection is more sensitive than FPD, because sulfur monoxide is the main combustion product [24]. Moreover, in contrast to FPD, it overcomes quenching phenomena due to the presence of carbon dioxide, water or hydrocarbons [24,27–29], and yields a linear response with increasing sulfur product concentration.

Two types of SCD exist: the SCD 350 and the SCD 355. The SCD 350 is coupled to a flame ionization detection (FID) instrument. The sulfur monoxide and other combustion products are formed in the FID flame [30]. It has the advantage of double detection (FID and SCD), while displaying a reduced sensitivity because of the positioning of the salvage probe and its relative lack of stability with time. The flameless SCD 355 is more sensitive. In fact, the probe is directly linked after the column to a combustion chamber and a regulator to control gas flows (hydrogen and air), the temperature and the pressure [31,32].

In this work, we further evaluated the capacity

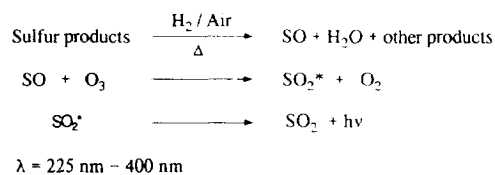


Fig. 1. Principle of chemiluminescence detection

of the SCD 355 and improved the analytical conditions using the static headspace technique. In that respect, several parameters were studied: the temperature, the ratio between the sample gas- and liquid-phase volumes, the liquid salt concentration (sodium chloride, ammonium sulfate and sodium sulfate) and the ethanol content.

2. Experimental

2.1. Quantitative analysis of sulfur products

All chromatograms were obtained using a Shimadzu GC 17 A gas chromatograph coupled to an SCD 355 chemiluminescence detector (Sievers). The detector signals were sent to a Kontron 450 MT2 data processing unit. Chromatographic separations were effected on a 30 m \times 0.32 mm I.D. polydimethylpolysiloxane (4 μ m film thickness) capillary column (SPB-1-Sulfur from Supelco).

The GC temperature programme was set as follows: 1 min at 35°C followed by a 10°C/min gradient to 55°C and then a 25°C/min gradient to 250°C. The injection temperature was 115°C.

In order to speed up the analytical separation and to enhance the sensitivity, pressure variations were used: 1 min at 82 kPa followed by a 30 kPa/min increase to 320 kPa. This shortened each analysis by 8 min and improved the peak detection and integration by yielding a better signal-to-noise ratio.

All gases used were highly pure (analytical-reagent grade). The hydrogen and air inputs into the burner were set at 100 and 40 ml/min, respectively. The oxygen pressure in the ozone generator was maintained at 48 kPa (7 p.s.i.g.).

Glass vials of 250 ml were used to generate the headspace. They were all tightly capped with silicone-rubber septa (sleeve stoppers).

Samples were obtained with a Hamilton 5-ml gas-tight Teflon Luer-Lock (1005 LTN). A 2-ml volume of the headspace volume was taken into the syringe before being adjusted to 1 ml 2 min later, and injected in the GC system with a splitting ratio of 1:5.

Ethyl methyl sulfide (EMS) was used as an internal standard. Calibration graphs were ob-

tained by plotting peak surface-area ratios, A (S-Pdts)/ A (EMS), versus concentration ratios, $[S-Pdts]/[EMS]$. EMS was elected as an internal standard for several reasons: it does not exist in the solutions to be tested, it does not react with the other compounds studied and it displays physico-chemical properties similar to those of the sulfur products under scrutiny.

2.2. Reagents and chemicals

Ethanol was of Normapur analytical-reagent grade, L-(+)-tartaric acid (TH2) and sodium sulfate were purchased from Prolabo, highly pure potassium hydrogentartrate (THK) from Merck, gaseous methanethiol (MeSH), diethyl sulfide (DES), ethanethiol (EtSH), thiophene, dimethyl disulfide (DMDS) and diethyl disulfide (DEDS) from Aldrich and dimethyl sulfide (DMS), ethyl methyl sulfide (EMS), carbon disulfide (CS₂), ammonium sulfate and sodium chloride from Fluka (all at least 97% pure). Sodium sulfide (Merck) was dissolved in aqueous solution buffered at pH 3.2 (TH2–THK) in order to generate hydrogen sulfide. Distilled, deionized water (resistivity 18.2 MΩ/cm) was used throughout. All brandies used were a generous gift from the Bureau National Inter-professionnel de Cognac (BNIC).

2.3. Solution preparation

Stock standard solutions of sulfur compounds (10–20 g/l) were prepared in ethanol to reduce losses by volatilization, and subsequently stored at 0°C until used. They were then diluted with cold ethanol or cold brandies in a cold room at 4°C to yield intermediate standard solutions at $4 \cdot 10^{-2}$ g/l that were kept at 0°C in closed test-tubes until used to prepare the final working standard solutions (concentrations and compositions are described in the figure legends). All standard solutions were stored in vials with headspace volumes as small as possible.

Water–alcohol (20:80) solutions were prepared by first dissolving 3 g/l of tartaric acid and 3 g/l of potassium hydrogentartrate (pH adjusted to 3.2) in water and by mixing in the desired volume of ethanol (medium 1). Medium

2 was obtained by diluting 70% brandy solutions to 20% ethanol and by adjusting the solution pH from 4.5 to 3.2. A given volume (V_L in Eq. 1) of these solutions was placed in 250-ml vials that were kept overnight at 4°C before addition of standard sulfur product (at 4°C).

$$V_L = V_1 + V_s \quad (1)$$

where V_L = total volume of the liquid phase, V_1 = volume of the water–alcohol solution or other medium and V_s = total volume of the introduced sulfur products.

All vials were hermetically capped with silicone-rubber septa (sleeve stoppers) and stored away from light in a thermostat-regulated water-bath before injection. The conditions used for the study of the various factors affecting the partition coefficient at a 50 μg/l concentration were a sample equilibrium temperature of 25°C with a partitioning equilibrium time of 72 h before injection.

3. Results and discussion

The performance of the detection of various substances with the headspace technique is generally higher in the dynamic than in the static mode [33]. However, the results obtained by the latter method are more realistically closer to the taster viewpoint, and the method is easier to set up. We therefore tried to improve the detection of volatile sulfur products obtained by the static headspace method. An SPB1-Sulfur column was used for the determination of volatile sulfur compounds at low concentration in the headspace [34].

3.1. Role of the water–alcohol medium for the partitioning of volatile compounds into the gas phase

Brandies are complex solutions and several substances in addition to water and ethanol can hinder the transfer of volatile sulfur products to the gas phase. We therefore followed the partitioning of nine sulfur compounds (among the most commonly found in brandies) from two

liquid phases: a pure synthetic water–alcohol solution and a commercial brandy solution (Figs. 2 and 3). Both of these solutions had similar pH (3.2) and ethanol content (20%).

It appears that, from brandy solutions only, the thiols (H_2S , EtSH, MeSH) cannot be detected in the gas phase even at concentrations up to $100 \mu\text{g/l}$, whereas they can easily (at $10 \mu\text{g/l}$ in the liquid phase) be detected if the liquid phase is a synthetic water–alcohol solution. Certain substances such as carbonylated compounds and heavy metals must exist in brandies that can interact with the thiols to lock them in the liquid phase.

3.2. Reliability of the detection and preparation method

The results in Table 1 show the reliability of the experimental protocol used for the study of

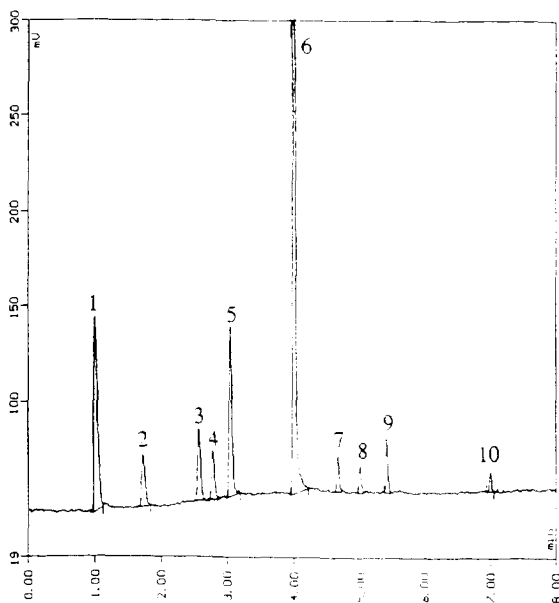


Fig. 2. Chromatograms of volatile sulfur compounds from medium 1. The initial concentration of each compound in the liquid phase was $10 \mu\text{g/l}$, except for EMS (internal standard), which was at $100 \mu\text{g/l}$. Peaks: 1 = H_2S ; 2 = MeSH; 3 = EtSH; 4 = DMS; 5 = CS_2 ; 6 = EMS; 7 = DES; 8 = thiophene; 9 = DMDS; 10 = DEDS.

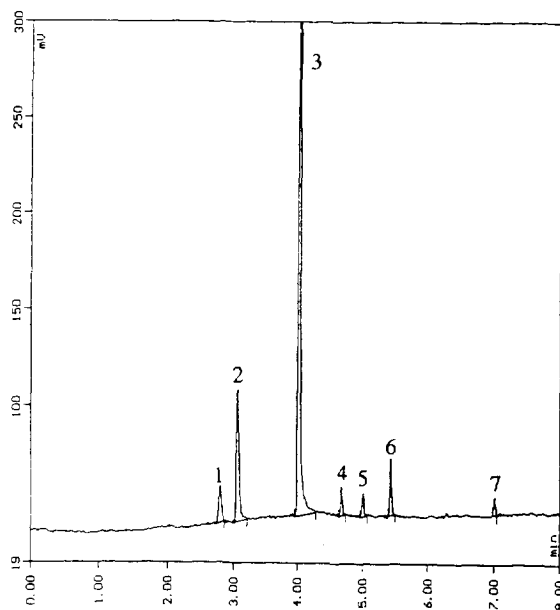


Fig. 3. Chromatograms of volatile sulfur compounds from medium 2. The initial concentration of each compound in the liquid phase was $10 \mu\text{g/l}$, except for the EMS (internal standard), which was at $100 \mu\text{g/l}$. Peaks: 1 = DMS; 2 = CS_2 ; 3 = EMS; 4 = DES; 5 = thiophene; 6 = DMDS; 7 = DEDS.

sulfur compounds in medium 1. In fact, the performances are similar for any sulfur compounds, except for thiols, which cannot be detected in medium 2. The repeatability and reproducibility of the method were determined at concentrations of 10, 20, 50 and $100 \mu\text{g/l}$. This concentration range was used because it covers the olfactory perception limit of most compounds in alcoholic beverages (0.02 – $100 \mu\text{g/l}$). After establishing the reliability of the method at $10 \mu\text{g/l}$, it was then necessary to determine the linearity of the detection response at concentrations up to $100 \mu\text{g/l}$.

Calibration graphs were calculated for H_2S , CH_3SH , $\text{C}_2\text{H}_5\text{SH}$, DMS, CS_2 , DES, thiophene, DMDS and DEDS in both media 1 and 2. All nine standards yielded a linear response in the concentration range 10 – $100 \mu\text{g/l}$ with an average correlation coefficient of 0.998. Typical calibration graphs for a thiol (EtSH), a sulfide (DES) and a disulfide (DEDS) are shown in Figs. 4–6.

Table 1
Determination of repeatability and reproducibility

Sulfur compound	Retention time (min)	A S-Pdts/A EMS ^a		Repeatability ^{b,c}		Reproducibility ^{b,d}	
		Medium 1	Medium 2	R.S.D. ₁ (%)	R.S.D. ₂ (%)	R.S.D. ₁ (%)	R.S.D. ₂ (%)
H ₂ S	1.00	0.362	ND ^e	1.20	–	1.27	–
MeSH	1.72	0.094	ND	1.71	–	4.31	–
EtSH	2.57	0.119	ND	1.94	–	1.54	–
DMS	2.78	0.081	0.077	1.34	1.32	1.48	1.64
CS ₂	3.06	0.320	0.300	2.71	1.70	4.51	2.32
DES	4.66	0.043	0.039	1.56	2.08	3.26	2.60
Thiophene	5.00	0.033	0.032	4.52	2.50	3.06	2.12
DMDS	5.43	0.06	0.069	3.67	2.32	4.02	2.32
DEDS	7.00	0.021	0.020	2.94	2.75	3.53	3.25

^a Mean of three values.

^b R.S.D. = relative standard deviation obtained with medium 1 and R.S.D.₂ = that obtained with medium 2.

^c The repeatability was obtained from three independent injections of the same sample.

^d The reproducibility was obtained using three independently prepared samples.

^e ND = not detected.

3.3. Influence of various physico-chemical factors on the partitioning of volatile sulfur products between a gas and a liquid phase

The liquid-to-gas volume ratio, V_l/V_g , has an influence on the detection of sulfur products. The results obtained are shown in Fig. 7 for a thiol, a sulfide and a disulfide, all with an ethyl group. The highest sensitivity is obtained for a V_l/V_g ratio of 4. This value was adopted for the study of the other parameters.

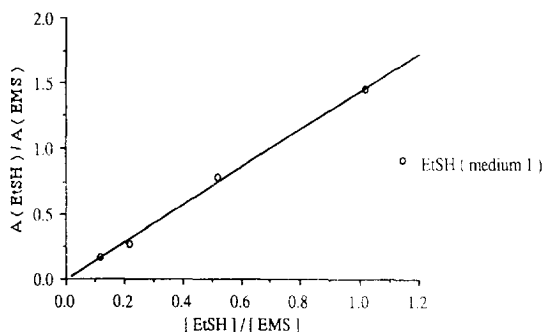


Fig. 4. Calibration graph for ethanethiol from medium 1. Each point represents the average value of three independent measurements. $y_1 = -3.3986 \cdot 10^{-2} + 1.4519x$; $r^2 = 0.997$.

Similar results were obtained with methyl-containing substances, hydrogen sulfide, carbon disulfide and thiophene. A closer look at the data, however, reveals that the value of the V_l/V_g ratio has a greater influence on sulfur products having a lower partition coefficient. A similar observation was made by Penton [35].

As described in the literature [36], the partition coefficient varies with temperature according to the Van't Hoff law [$\log K_i = f(1/T)$]. We

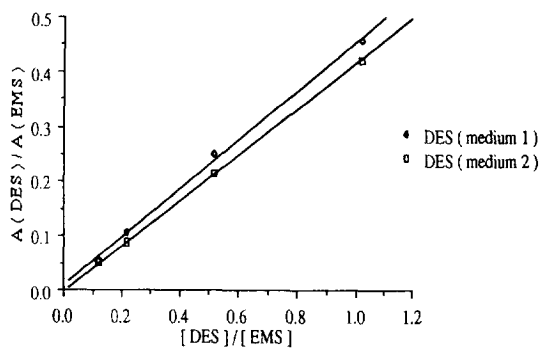


Fig. 5. Calibration graph for diethyl sulfide from media 1 and 2. Each point represents the average value of three independent measurements. $y_1 = 5.918 \cdot 10^{-3} + 1.4519x$; $r^2 = 0.998$. $y_2 = -5.0116 \cdot 10^{-3} + 0.41416x$; $r^2 = 1.000$.

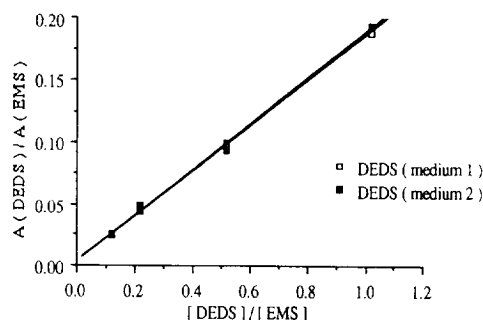


Fig. 6. Calibration graph for diethyl disulfide from media 1 and 2. Each point represents the average value of three independent measurements. $y_1 = 2.7016 \cdot 10^{-3} + 0.18174x$; $r^2 = 1.000$. $y_2 = 2.528 \cdot 10^{-3} + 0.18395x$; $r^2 = 0.997$.

therefore followed the temperature-dependent variations in partition coefficient in order to find the optimum analytical conditions in terms of detection. After leaving samples for 72 h at 25°C away from light, they were brought to 40, 60 and 80°C and analysed 2 h later.

The results obtained with ethanethiol, diethyl sulfide and diethyl disulfide on medium 1 and diethyl sulfide and diethyl disulfide on medium 2 are displayed in Figs. 8 and 9, respectively. They show linear increases in detectability with increasing temperature. However, a standard temperature of 25°C was selected for samples analysis because it better reflects the olfactory sensations of the taster. Different sulfur products exhibit different temperature dependences. For

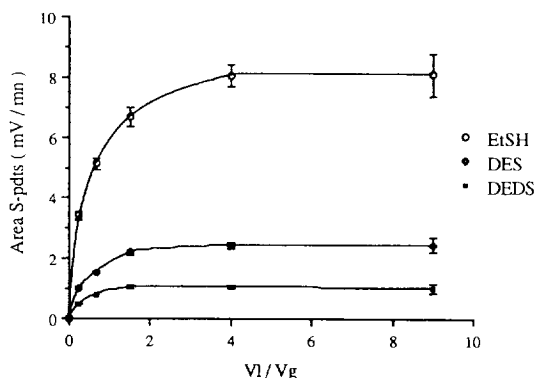


Fig. 7. Influence of the volume ratio V_l/V_g on partitioning of sulfur compounds in the liquid and gas phases. Each point represents the average value of three independent measurements.

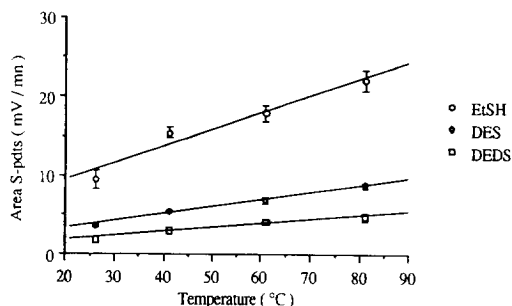


Fig. 8. Dependence on the gas concentration of volatile sulfur compounds from medium 1 on equilibrium temperature. Each point represents the average value of three independent measurements. $y_{\text{EtSH}} = 4.5242 + 0.21369x$; $r^2 = 0.951$. $y_{\text{DES}} = 0.73470 + 9.2038 \cdot 10^{-2}x$; $r^2 = 0.991$. $y_{\text{DEDS}} = -5.4556 \cdot 10^{-2} + 5.3666 \cdot 10^{-2}x$; $r^2 = 0.971$.

example, ethanethiol, which is more hydrophilic and alcoholophilic at 80°C, displays larger temperature-dependent variations than sulfides and disulfides.

Among the many physico-chemical factors that influence the detection of sulfur products, the ionic strength of the liquid phase was studied in order to alleviate the matrix effect encountered in brandies. For this purpose, medium 2 was modified with increasing sodium chloride, sodium sulfate or ammonium sulfate concentration. Surprisingly, Fig. 10 shows that increasing sodium chloride concentrations do not modify the response of thiols and hydrogen sulfide, whereas

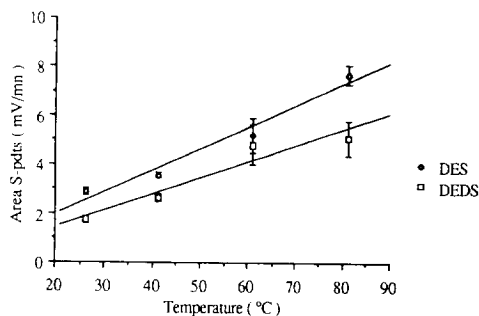


Fig. 9. Dependence on the gas concentration of volatile sulfur compounds from medium 2 on equilibrium temperature. Each point represents the average value of three independent measurements. $y_{\text{DES}} = 6.24054 \cdot 10^{-2} + 8.8197 \cdot 10^{-2}x$; $r^2 = 0.967$. $y_{\text{DEDS}} = -4.1406 \cdot 10^{-2} + 6.5751 \cdot 10^{-2}x$; $r = 0.921$.

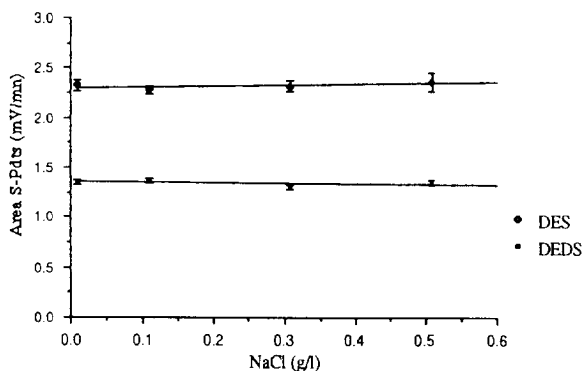


Fig. 10. Effect of increasing amounts of dissolved salts on the liquid-gas partitioning of volatile sulfur compounds from medium 2. Each point represents the average value of three independent measurements.

they affect those of the less hydrophilic sulfides and disulfides. Similar results were obtained with other salts such as sodium sulfate and ammonium sulfate.

Przyjazny et al. [36] reported that for sulfur products the partition coefficients increase and hence their gas concentrations decrease with increasing solubility in water at 25°C. Such a result prompted us to evaluate the detectability of the standards used in the headspace according to the ethanol content of the liquid phase in water-alcohol solutions and brandies.

The results in Figs. 11 and 12 clearly show that a constant increase in ethanol content leads to a

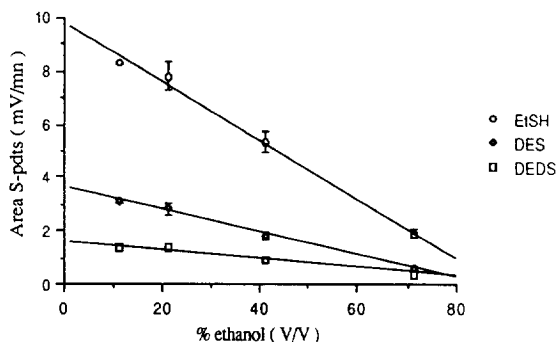


Fig. 11. Effect of increasing amounts of ethanol in water-alcohol solution. Each point represents the average value of three independent measurements. $y_{\text{EISH}} = 9.4250 - 0.11026x$; $r^2 = 0.992$. $y_{\text{DES}} = 3.3289 - 4.2778 \cdot 10^{-2}x$; $r^2 = 0.995$. $y_{\text{DEDS}} = 1.3283 - 1.67380 \cdot 10^{-2}x$; $r^2 = 0.980$.

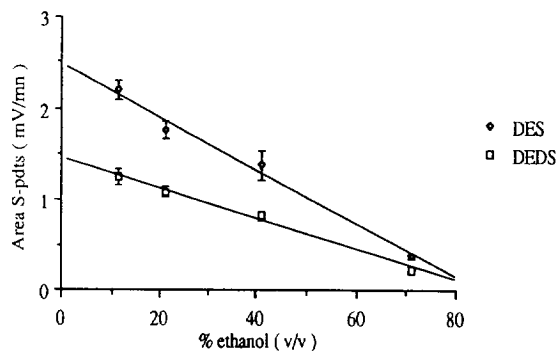


Fig. 12. Effect of increasing amounts of ethanol in brandy solution. Each point represents the average value of three independent measurements. $y_{\text{DES}} = 2.3809 - 2.9315 \cdot 10^{-2}x$; $r^2 = 0.989$. $y_{\text{DEDS}} = 1.3634 - 1.6851 \cdot 10^{-2}x$; $r^2 = 0.990$.

gradual and linear decrease in the amount of product detected. In order to minimize this “ethanol effect” in brandies, the ethanol content was decreased to 20% by adding distilled, deionized water. This choice was made as a compromise between a lower ethanol effect and a not too large dilution of the samples.

4. Conclusion

The results presented in this paper illustrate the high performance of the Sievers SCD 355 detector. Its direct connection to the output of the column, without an intermediate FID instrument such as is used with the SCD 350, makes it more stable with time and results in higher reliability in terms of repeatability and reproducibility. Moreover, it exhibits a linear response over a wide concentration range, in contrast to the FPD instrument. We have also demonstrated that the static headspace technique is simple and reliable.

Several new pieces of information have originated from this work: (i) the linearity and repeatability of the SCD 355 detector and the reproducibility of the sample preparation; (ii) the importance of the V_l/V_g ratio, which yields optimum detection at a value of 4; (iii) the role of the ethanol content; (iv) the addition of salt has no effect on the release of thiols from the

liquid phase; and (v) even though increasing temperatures enhance the response much more than a change in the above parameters, we decided to work at 25°C in order to prevent undesirable reactions between sulfur products or with the electrophilic substances present in complex solutions (wines, brandies, etc.) and to reflect better the olfactory impression obtained during tasting. This choice of temperature will not be a limitation if the chromatographic unit is equipped with a pressure gradient system, which greatly shortens the retention times while lowering the detection limit.

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