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Original Article

Identification of trace volatile compounds in freshly distilled Calvados and Cognac: Carbonyl and sulphur compounds

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Abstract

Carbonyl and sulphur compounds were investigated in freshly distilled Calvados and Cognac. Forty-eight aldehydes and ketones were identified in both products using specific extraction, with Girard's T reagent supported on silica-gel. The recovery procedure of these compounds in a high ethanolic medium was optimized. Samples contained a majority of saturated aliphatic aldehydes and ketones. The presence of unsaturated aliphatic aldehydes and ketones, such as alkenals, oct-1-en-3-one, 6-methylhept-5-en-2-one and β-damascenone, was also detected. Numerous terpenoic or norisoprenoic carbonyl compounds were characterized and seem to be specific to Cognac. Twenty-seven sulphur compounds were quantified in the same samples; small amounts of thiols were detected. Concentrations of dimethyl disulphide were from 50–500 μg L^{-1} of pure alcohol (PA), and a high content of dibutyl sulphide (20–100 μg L^{-1} of PA) was recorded. Concentrations of ethyl propyl sulphide and diethyl sulphide, which were not significant in Calvados, were above 25 μg L^{-1} of PA in Cognac. 2- and 3-thiophenecarboxaldehyde were also quantified, and concentrations as high as 1000 μg L^{-1} in some samples, were the most important in Cognac. The ratio of 3- and 2-thiophenecarboxaldehyde was higher than 10 for Calvados, but was less than 0.5 for Cognac. Although weak concentrations of methional were recorded, this may contribute to the overall aroma of these two spirits due to extremely low detection threshold. The total concentration of sulphur compounds in Cognac samples was five to six times that of Calvados samples.

Keywords: Calvados; Cognac; Carbonyl compounds; Sulphur compounds; GC/MS; GC/SCD; Girard's T reagent

1. Introduction

This work was carried out within the framework of a French national programme undertaken jointly by various research teams. It was performed with the purpose of controlling spirits technology, and therefore freshly distilled samples of good-quality spirits were specifically selected. In a preliminary study, more than 300 volatile compounds were identified in both types of spirits (Ledauphin et al., 2004). Among these, only a few carbonyl and sulphur compounds were detected. However, olfactometric studies (Ledauphin et al., 2003; Ferrari et al., 2004) revealed their high olfactometric impact, even at low concentrations. Our goal was therefore to identify the carbonyl compounds that could be responsible for olfactive defects or quality, and also to quantify sulphur compounds in order to evaluate their presence and organoleptic effects. This study is a prospective work on carbonyl and sulphur compounds present in freshly distilled Calvados and Cognac, the goal of which is to enhance a few constituents to be followed during the technological process to improve quality of the final products.

Calvados is a typical spirit produced in Normandy. Freshly distilled Calvados results from the distillation of cider. The French label "AOC" (*Appellation d'origine controlée*) is given immediately after the distillation step. The quality of Cognac spirits is recognized throughout

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the world. The geographic area used for Cognac production in France includes nearly all of Charente-Maritime, a very large part of Charente and a few neighbouring villages. The wine distilled in order to obtain Cognac spirits originates from specific vine varieties (mainly *Ugni blanc* today). A few studies (Guichard et al., 2003; Ledauphin et al., 2003; Ledauphin et al., 2004; Ferrari et al., 2004) have been carried out to determine the presence of the volatile components and key odorants of these two freshly distilled spirits. Although only a few carbonyl and sulphur compounds were identified, these can produce important organoleptic defects. In this study, we have attempted to identify them in freshly distilled Calvados and Cognac and to quantify the sulphur compounds in both spirits.

1.1. Carbonyl compounds

Aldehydes and ketones can contribute to unpleasant green notes in wine (Hashizume and Samuta, 1997) and also in distilled spirits like whisky (Wanikawa et al., 2002). In aged spirits, many of these compounds, such as vanillin, coniferaldehyde, sinapaldehyde or syringaldehyde, are known to come from wood tannins (Puech et al., 1994; Gimenez Martinez et al., 1996). Nevertheless, numerous carbonyl compounds have been reported as being part of the volatile composition of freshly distilled Calvados (Ledauphin et al., 2003, 2004), whisky (Kahn et al., 1969) and various brandies or spirits (Barroso et al., 1996; Cardoso et al., 2003).

The most predominant aldehyde in freshly distilled beverages is acetaldehyde, but the contribution of this compound to organoleptic quality has been investigated intensively (Nykänen, 1986). Among acetaldehyde, the main aldehydes identified were: acrolein, which is known to give rise to an unpleasant pungent odour; 2,3-butanedione with a buttery aroma, which was identified in nearly all spirits and also furfural. With the exception of these compounds, aldehydes and ketones in general show very low concentrations in distilled spirits. Nevertheless, because of their very low detection threshold, they can produce an important olfactive impact (Bylaite et al., 2003). As carbonyl function is moreover very reactive, an evaluation of these compounds can be strongly depreciated by reaction with the matrix. Conversion of aldehydes into acetals was notably reported by using liquid–liquid extraction with polar solvents in highly ethanolic media. As a consequence, because of their relative instability in complex media, carbonyl compounds usually undergo a derivatization step prior to analysis. The most common method improved the reactivity of 2,4-dinitrophenylhydrazine (2,4-DNPH).

Derivatives were analysed by high performance liquid chromatography (HPLC) coupled with either UV detection (Fung and Grosjean, 1981), diode array detection (Cardoso et al., 2003) or mass spectrometric detection (Grosjean et al., 1999; Possanzini et al., 2000). In the particular case of low weight molecular carbonyl compounds derivatives, gas chromatography (GC) was also used and was coupled with a mass spectrometer (Dong and Moldoveanu, 2004), an electron capture detector or a flame ionization detector (Velikonja et al., 1995).

In the past few years, numerous other methods of derivatization of carbonyl compounds in complex media have been developed. For instance, aldehydes and ketones were transformed into oximes using *o*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (Le Lacheur et al., 1993; Ojala et al., 1994) or into thiazolidines using cysteamine (Yasuhara and Shibamoto, 1995; Yasuhara et al., 1998). However, these methods of derivatization produce less volatile compounds, and this decreases the opportunity of characterizing aldehydes and ketones by GC/MS.

In this study, we chose to isolate specifically trace volatile carbonyl compounds from the matrix in order to identify them directly by GC/MS. Once these compounds are isolated in an organic solvent selected for its high volatility, a soft evaporation can be applied to obtain important concentrations of aldehydes and ketones in small volumes. As a consequence, we were able to identify them in Calvados and Cognac using Girard's T reagent. This reagent has already been employed for the isolation of numerous natural compounds (Wheeler, 1962, 1968) and utilizes a reaction with aldehydes and ketones to produce the corresponding hydrazones. The method was improved (Singh et al., 1981) in the early 1980s by the adsorption of the reactant on a solid support, and selective extraction of carbonyl compounds from essential oils was performed. Experimental procedures were adapted to the analysis of carbonyl compounds in highly alcoholic media by using an ethanol/water mixture, since this type of extraction was not used in the past for highly ethanolic liquid matrices. Each step of the extraction process was optimized in order to recover a maximum yield of aldehydes and ketones from a hydroalcoholic medium.

1.2. Sulphur compounds

Sulphur compounds are known to be responsible for numerous aromatic defects in beverages. 2-Mercaptoethyl acetate and 3-mercaptopropyl acetate in particular play a significant role in the "grilled" aroma of wines (Lavigne et al., 1998). Nevertheless, more than 40 sulphur compounds have been detected in wines, with odours that can be described as cabbage, garlic, onion, cooked or sulphurous (Mestres et al., 2000). In general sulphur compounds have very low detection thresholds. As a result, it is usually difficult to characterize them using the more popular detection systems (flame ionization or mass spectrometry) in GC. They are now easily detected or quantified using specific detectors: flame photometry (FPD) (Darriet et al., 1995; Mestres et al., 1998, 1999) or sulphur chemiluminescence (SCD) (Nedjma and Maujean, 1995). Extractions are commonly carried out by liquid–liquid extraction, static headspace, dynamic headspace or, more recently, by solid-phase microextraction (Mestres et al., 2000). In this paper, quantification of about 30 sulphur compounds was carried out via GC/SCD in freshly distilled Calvados and Cognac.

2. Materials and methods

2.1. Materials

The sensory characteristics of a dozen of samples of freshly distilled Cognac (Ferrari et al., 2004) and a dozen of samples of freshly distilled Calvados (Ledauphin et al., 2004) were tested by a professional panel, by nose and in mouth. Produced in their respective limited AOC regions in 2000–2001, these samples were provided voluntarily, and selected in-house by the producers themselves, as "good quality" spirits. Two samples of each spirit, presenting the more pleasant organoleptic characteristics, were then selected for this study. The two samples of Calvados (from the French region named "Calvados") were labelled Calvados 1 and 2, and the two samples of Cognac (from the French region named "Cognac") were labelled Cognac 1 and 2. Solvents (supplied from Acros Organic, New Jersey) with HPLC grades were submitted to a double-distillation as follows: pentane, toluene and diethyl ether.

2.2. Identification of carbonyl compounds: procedure

2.2.1. Preparation of silica-gel supported Girard's T reagent

A quantity of 10 g of Girard's T reagent trimethylammonium acetyl hydrazide chloride from Sigma-Aldrich was added to 90 g of silica-gel (0.063–0.200 mm granulometry from Merck, Darmstadt, Germany), then poured in a mixture containing 80 mL of propan-2-ol and 80 mL of ultra-pure water. The mixture was vigorously stirred at room temperature for two hours in a 500 mL corked round-bottom flask. Solvent was removed with a rotary evaporator. A fine powder was obtained and stored in a dark, light-free place.

2.2.2. Extraction of organic compounds

A quantity of 100 mL of freshly distilled Calvados or Cognac was diluted with 200 mL of ultra-pure water in a 500 mL Erlenmeyer flask. Next 20 g of NaCl, 2 mL of NaOH (0.5 M), and then 15 mL of toluene (or pentane for the experiments in Fig. 1) were successively added to the mixture, which was stirred for 10 min under magnetic agitation. The mixture was poured into a separator funnel and the organic layer was recovered. This process was repeated three times, leading to the recovery of 50 mL of cumulated extract of toluene, which contained the organic volatile molecules. The organic layer was finally dried on magnesium sulphate and filtered through deactivated glass wool.

2.2.3. Condensation of carbonyl compounds on solid-phase

The organic layer was poured into a 100 mL round bottom flask equipped with a reflux condenser. 20 g of silica-gel containing Girard's T reagent were added, and the mixture was heated at 70 °C overnight under vigorous magnetic agitation.

2.2.4. Solid-phase recovery

The mixture obtained was made up of a liquid layer and a solid-phase containing condensed aldehydes and ketones. Silica-gel was isolated and dried by pouring the mixture through a fitted filter funnel coupled to a filter flask. The silica-gel was rinsed 10 times using 30 mL of toluene to eliminate non-carbonyl compounds. A very

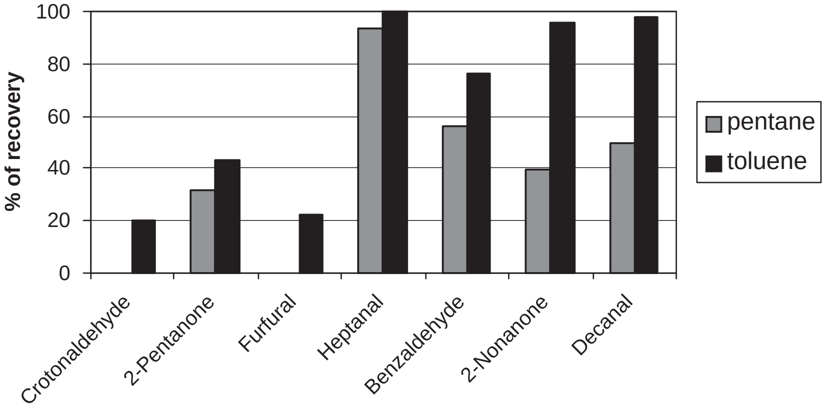

Fig. 1. Recovery of some carbonyl compounds after liquid–liquid extraction using toluene and pentane.

dry fine white powder was obtained at the end of this step.

2.2.5. Recovery of carbonyl compounds after hydrolysis

Silica-gel was poured into a 100 mL round-bottom flask into which 20 mL of diethyl ether followed by 30 mL of HCl (2 M) were added. The mixture was vigorously stirred overnight under magnetic agitation. The solvent was recovered by means of decantation. 15 mL of diethyl ether were added three times in order to wash the silica-gel, followed by 10 min of decantation. The 60 mL extract obtained was dried on magnesium sulphate.

2.2.6. Reduction on Kuderna– Danish column

The extract was reduced by evaporation of solvent to a final volume of 300 μL using a Kuderna–Danish column. It was stored at −20 °C prior to analysis on GC/MS.

2.3. Identification of carbonyl compounds: GC/MS

GC/MS analyses were carried out on a Varian 3800 gas chromatograph interfaced with a Saturn 2000 mass spectrometer. Separations were performed using a 30 m × 0.25 mm (i.d.) capillary column coated with a 0.15 μm film of ZB-Wax stationary phase (100% polyethyleneglycol from Phenomenex, Torrance, CA), which is equivalent to DB-Wax or Carbowax 20 M. Helium was used as a carrier gas with a 1 mL min^{-1} flow rate. For the analysis of the extracts, the oven programme temperature used was 35–220 °C at a rate of 5 °C min^{-1}, with an initial temperature hold for 5 min and a final temperature hold for 10 min, resulting in a total run of 52 min.In total, 1.5 μL of extracts were injected in splitless and split mode 100:1, and injection port temperature was fixed at 250 °C.

For each extract, the mass spectrometer, equipped with an ion trap analyser, was operated in electron impact. Ionization voltage was 70 eV, ion source temperature 150 °C and electron multiplier voltage 1350 V. Scanning was performed on a range from m/z 35–400.

For each peak detected, a linear retention index (R.I.) was calculated using GC R.I. internal standards (hydrocarbons from C7 to C31) according to the method of Van den Dool and Kratz (Van Den Dool and Kratz, 1963).

The identification of volatile compounds was performed principally by a comparison of the mass spectra obtained with those of the NIST 98 MS database or of a "home-made" database. Theoretical linear indexes were also calculated for compounds belonging to same chemical classes and compared to indexes of unknown chromatographic peaks. Aldehydes and ketones identified in each of the samples are reported in Table 4. Results are compared to that obtained in a previous study where liquid–liquid extraction on the same samples was carried out using dichloromethane (Ledauphin et al., 2004).

2.4. Identification of carbonyl compounds: method development

Material: The experimental procedure was optimized by using a standard solution (see Table 1), which contained eight carbonyl compounds. Three other volatile compounds were also added in order to check specificity. Concentrations of these were of 100 mg L^{-1} in pentane or toluene. Analyses were carried out on a Varian 3300 Gas Chromatograph equipped with a flame ionization detector heated to 240 °C. Injections were made in split mode with a split ratio of 1:100. Injection volumes were 1 μL. The injection port temperature was 240 °C. Nitrogen was used as a carrier gas with a

Table 1
Composition of the carbonyl compounds standard solution used for the optimization of the experimental procedure and total recovery after reaction with Girard's T reagent

Compound[a]	Chemical class	MW (g mol^{-1})	Total recovery (%)
3-Methylbutanol	Alcohol	88.1	0
2-Methylbutanoic acid	Carboxylic acid	102.1	0
Ethyl lactate	Hydroxyester	118.1	0
Crotonaldehyde (but-2-enal)	Unsaturated aldehyde	70.1	1
3-Methylbutanal	Ramified aldehyde	86.1	77
Heptanal	Aliphatic linear aldehyde	114.2	100
Decanal	Aliphatic linear aldehyde	156.3	82
Pentan-2-one	Aliphatic linear ketone	86.1	69
Nonan-2-one	Aliphatic linear ketone	142.2	82
Furfural	Furanic aldehyde	96.1	8
Benzaldehyde	Aromatic aldehyde	106.1	88

[a] Supplied by Acros Organics; concentrations: 100 mg L^{-1} for each.

1 mL min^{-1} flow rate. Separations were performed using a 25 m × 0.22 mm i.d. capillary column, coated with a 0.25 μm film of BP-10 stationary phase [(5% diphenyl/ 95% dimethyl)polysiloxane from Supelco, Bellefonte, PA]. The oven temperature programme ranged from 35 to 210 °C at a rate of 10 °C min^{-1}, with an initial temperature hold for 10 min and a final temperature hold for 2 min, resulting in a total run of about 30 min. Data were recorded with a Varian 4400 integrator.

Extraction of carbonyl compounds: 100 mL of ethanol/ water (70/30) mixture were diluted with 200 mL of ultra-pure water in a 500 mL Erlenmeyer. 20 g of NaCl, 2 mL of NaOH (0.5 M) and 30 mL of standard solution (see Fig. 1) in toluene or pentane were successively added to the mixture, which was stirred for 10 min under magnetic agitation. The mixture was poured into a separator funnel and the organic layer was recovered; 3 μL ethyl undecanoate used as internal standard were added not only to the extract before injection in GC/ FID, but also to 30 mL of standard solution for comparison. The relative area of each peak was calculated in organic extracts and then compared to that of the standard solution. The recovery ratios are reported in Fig. 1.

Kinetics of reaction: 5 μL of ethyl undecanoate were added to 50 mL of toluene containing 100 mg L^{-1} of heptanal and nonan-2-one. The chromatogram of this solution, injected in GC/FID, was used as a reference. This solution was poured into a 100 mL round-bottom flask equipped with a reflux condenser. A total of 20 g of silica-gel containing Girard's T reagent were added and the mixture was heated at 70 °C. Samples of a few microlitres of the medium were taken at 15 min, 1 h, 1 h30, 2 h, 4 h, 6 h and 8 h. Then 1 μL of each was injected into GC/FID and compared with the initial solution. Results of kinetics are reported in Fig. 2.

Recovery of volatile compounds after reaction with Girard's T reagent supported on silica-gel: 50 mL of standard solution were used (see Table 1). The condensation of carbonyl compounds with Girard's T reagent followed by recovery after hydrolysis was carried out according to the procedure described above. Diethyl ether volume was finally adjusted to 50 mL and 5 μL of ethyl undecanoate added to the extract. In total, 1 μL was injected into GC/FID and compared to the initial standard solution containing the internal standard. The results of this step are also shown in Table 1.

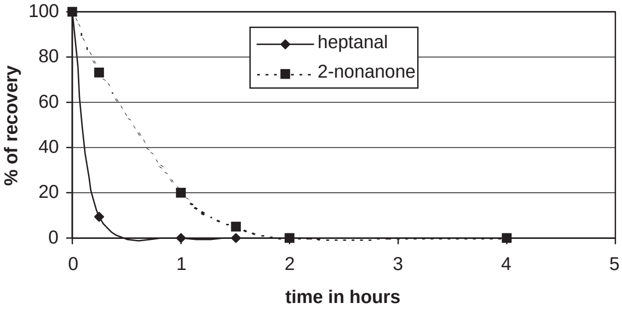

Fig. 2. Kinetics of reaction of Girard's T reagent with heptanal and nonan-2-one.

2.5. Quantification of sulphur compounds

Chemicals: Standard solutions were prepared in anhydrous ethanol; these were available from commercial high-purity sulphur compounds, which were not further purified before use. Five different solutions (A–E) containing 4–7 compounds were prepared from pure sulphur compounds in anhydrous ethanol by dilution in a 70/30 ethanol/water mixture. They all contained thiophene (from fluka) as an internal standard, with a concentration of 6.34 mg L^{-1}. The characteristics of each sulphur compound (purity, supplier, concentration) and the solutions to which they were added are indicated in Table 2. They were purposely not collected from the same solution so that identification would be easier. The retention times given in Table 3 are relative to thiophene (the internal standard present in all solutions). The identification of each sulphur compound detected in freshly distilled Calvados and Cognac was then completed by a comparison of relative retention times (compound retention time/thiophene retention time).

Extraction of sulphur compounds: 100 mL of freshly distilled Calvados or Cognac (70% v/v) were diluted with 200 mL of ultra-pure water. A total of 10 mL of thiophene (used as internal standard) with a concentration of 6.33 mg L^{-1} in pure ethanol were added to the mixture, with 15 g of NaCl. Sulphur compounds were extracted with 16 mL of dichloromethane and stirred for five min at room temperature. The mixture was poured into a separator funnel, and the organic layer was recovered.

Chromatography: Analyses were carried out on an HP 5890 gas chromatograph equipped with a Sievers 355B model sulphur chemiluminescent detector (SCD). Separations were performed using a 30 m × 0.32 mm (i.d.) capillary column, coated with a 4 μm film of SPB-1 apolar stationary phase (from Supelco, Bellefonte, PA, USA). The oven temperature programme used was 35–210 °C at a rate of 10 °C min^{-1}, with an initial temperature hold for 1 min and a final temperature hold for 15 min. Injections were made in splitless mode for 1 min and total flow was 25mL min^{-1}. Injection volumes were 1 μL. The injection port temperature was 120 °C and the detector temperature was 220 °C. Helium was used as a carrier gas with a 1.4 mL min^{-1} flow rate.

Quantification: Linear detection of sulphur compounds was verified using GC/SCD and quantification was performed by calculation of the response factors of each. The relative area of each peak enabled the

Table 2
Composition of standard solutions in sulphur compounds

Compound[a,b,c]	Solution no.	Molecular weight (g mol^{-1})	Purity (%)	Concentration (mg/L^{-1} of PA)
Dimethyl sulphide	1	62.1	99	0.96
Ethanethiol	2	62.1	97	0.73
Propane-1thiol	3	76.2	97	0.57
Methyl ethyl sulphide	4	76.2	97	0.76
Diethyl sulphide	1	90.2	98	0.72
2-Methylpropane-1-thiol	5	90.2	90	0.56
Butane-1-thiol	3	90.2	95	1.81
Butane-2-thiol	2	90.2	95	0.38
2-methylthioethanol	5	92.2	98	0.79
Dimethyl disulphide	1	94.2	98	0.83
Ethyl propyl sulphide	2	104.2	98	1.00
3-Methylthiopropanal[a]	2	104.2	100	1.08
Pentanethiol[a]	3	104.2	98	0.58
3-Methylthiopropanol[a]	3	106.2	98	0.75
2-Thiophenecarboxaldehyde[a]	2	112.1	98	0.61
3-Thiophenecarboxaldehyde[a]	3	112.1	98	0.54
Thiophenol[b]	5	118.2	99	0.81
Dipropyl sulphide	1	118.2	90	0.25
4-Methylthiobutanol[a]	5	120.2	90	0.36
Diethyl disulphide[c]	1	122.2	98	0.87
Benzothiazole[a]	4	135.2	96	0.65
Diethyl sulphite	4	138.9	98	0.67
Dibutyl sulphide	1	146.3	95	0.31
Dipropyl disulphide	1	150.3	97	0.49
3-Methylthiopropyl acetate	4	148.2	98	0.31

Supplier: all compounds from Fluka except (a) Aldrich, (b) Prolabo, (c) Sigma.

determination of concentrations of each sulphur compound in the Cognac and Calvados samples. The results presented in Table 3 (containing all studied sulphur compounds labelled from 1 to 28) are expressed in µg of compound per litre of pure alcohol (PA).

3. Results and discussion

3.1. Carbonyl compounds

Our goal was to isolate carbonyl compounds from the natural hydroalcoholic medium by means of a selective concentration. A certain number of reagents were used for their specificity relative to aldehydes and ketones, among which Girard's T reagents (Girard and Sandulesco, 1936; Wheeler, 1962, 1968) have proved to be relevant. These quaternary ammonium hydrazides react with the carbonyl function to give a hydrazone, which can be linked to an inorganic polymer by simple adsorption (see Fig. 3). The relevance of Girard's T-on-silica gel reagent (Singh et al., 1981) lies in the handling of a solid matrix, which can be easily isolated or washed and makes it possible to work on a large scale. As a consequence, a high concentration of aldehydes and ketones can be recovered after hydrolysis from samples in which these compounds may occur only in trace quantities. However, in our case, a precise and meticulous optimization had to be achieved because the excess of ethanol in the sample matrix, and the presence of polyfunctionalized carbonyl compounds with various polarities, can occur. Each step of this process of recovering specific carbonyl compounds was optimized.

First, liquid–liquid extraction of carbonyl compounds was studied using two different solvents. The use of polar solvents like methanol or dichloromethane was avoided because of their high affinity with silica-gel. Pentane and toluene were tested with a standard solution (see Table 1). The results presented in Fig. 1 show that recovery of non-polar aldehydes and ketones was quantitative using toluene. Extraction of polar compounds like crotonaldehyde and furfural was more difficult, but it was possible to recover at least 20% of each with a single extraction. The kinetic of adsorption of carbonyl compounds was also followed using heptanal and 2-nonanone. Heptanal was rapidly converted into the corresponding silica-gel supported hydrazone (see Fig. 2), whereas the kinetic of reaction of ketones seemed to be slower. Nevertheless, conversion was completely achieved in a few hours.

After hydrolysis, carbonyl compounds were recovered, and the ratio of each one was determined with regard to the standard amount treated (see Table 1). Results obtained after this last step showed that the method is specific to carbonyl compounds. Indeed, 3-methylbutanol, ethyl lactate and 2-methylbutanoic

Table 3
Results of sulphur compounds quantification in samples of freshly distilled Cognac or Calvados

Entry	Sulphur compound	Detection thresholds ($\mu g\,L^{-1}$)	Odour[a]	Retention time (min)	Concentrations ($\mu g\,L^{-1}$ of PA)				DL_{50}[c] (mg/kg)
					Cognac 1	Cognac 2	Calvados 1	Calvados 2	
1	Ethanethiol	1.1[a]	Onion, rubber[a]	3.53	0	0	0	0	682
2	Dimethyl sulphide	10–160[a]	Cabbage, asparagus[a]	3.79	Traces	Traces	traces	Traces	535
3	1-Propanethiol	3.1[b]		5.94	0	0	0	0	1790
4	Methyl ethyl sulphide			6.06	0	0	0	0	
5	2-Butanethiol			7.26	0	0	0	0	
6	Thiophène (I.S.)			7.36	633	633	633	633	
7	2-Methyl propanethiol			7.57	23	0	0	0	
8	Diethyl sulphide	0.93–18[b]	Garlic[a]	8.08	0	0	3	1	
9	1-Butanethiol	6[b]		8.39	17	0	0	0	1500
10	Dimethyl disulphide	20–45[a]	Cabbage, cooked cabbage[a]	9.13	498	73	119	57	290–500
11	Ethyl propyl sulphide			10.37	42	33	3	Traces	
12	1-Pentanethiol			10.86	1	0	0	0	
13	Methyl ethyl disulphide			11.23	10	0	1	1	
14	2-Methylthioethanol	250[a]	French bean, bean[a]	11.71	13	0	6	Traces	
15	Diethyl sulphite			12.09	25	53	0	0	
16	3-Methylthiopropanal	0.2[b]	Onion, meat, mashed potatoes[a]	12.31	39	0	11	10	4400
17	Dipropyl sulphide			12.53	2	0	0	0	
18	Diethyl disulphide	4.3–40[a]	Bad smelling, onion[a]	13.27	0	0	4	4	
19	3-Methylthiopropanol	1200–4500[a]	Potato, soup[a]	14.02	Traces	Traces	Traces	Traces	
20	Thiophenol	100–500[a]		14.14	4	0	12	8	46.2
21	3-Thiophenecarboxaldehyde			14.33	564	342	190	321	
22	2-Thiophenecarboxaldehyde			14.57	1207	1233	14	22	915
23	Ethyl propyl disulphide			15.24	122	6	18	22	
24	Dibutyl sulphide			16.58	97	21	56	44	
25	3-Methylthiopropyl acetate	50–115[a]	Mushroom, garlic[a]	16.84	30	14	28	32	
26	4-Methylthiobutan-1-ol	100[a]	Onion, garlic[a]	16.93	0	91	0	0	
27	Dipropyl disulphide			17.08	36	5	1	1	
28	Benzothiazole	50–350[a]	Rubber[a]	19.48	13	17	8	20	479
	Total concentration of identified sulphur compounds				2743	1888	474	543	

[a] References reported from Mestres et al. (2000), odours thresholds calculated in wines.

[b] References reported from ''Leffingwell flavour-base 2004 demo'' (http://www.leffingwell.com), odours thresholds calculated in water.

[c] Oral half lethal doses for rats reported from Merck Chemical Database (http://www.chemdat.de).

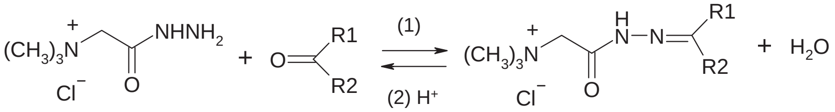

Fig. 3. Reaction of Girard's T reagent with carbonyl compounds (1) and hydrolysis of the hydrazone (2).

acid—which belong to the alcohol, ester and carboxylic acid chemical classes—were not recovered at the end of the process. This method is not well optimized for the characterization of most polar aldehydes, firstly because their extraction is rather difficult, and secondly because side-reactions of unsaturated conjugated carbonyl compounds with Girard's T reagent may produce 1,4 Mickael addition-type adducts which cannot be further hydrolysed. Nevertheless, recovery of a part of polar aromatic compounds such like furfural or benzaldehyde can be performed. Moreover, recovery of apolar carbonyl compounds is quite quantitative.

A total of 21 aldehydes and 27 ketones, labelled from 1′ to 48′ in Table 4, were identified in both samples of Cognac and Calvados. In spite of their relatively low concentrations, about 25 carbonyl compounds can be detected for each sample. Only six aldehydes and ketones had already been identified in the same four samples (Ledauphin et al., 2004) after a liquid–liquid extraction using dichloromethane, followed by a soft evaporation of the solvent. This substantial difference can be explained above all by the factor of concentration of the sample. The isolation of carbonyl compounds by our present method enables one to reduce the volume from 100 mL of sample to a 300 μL extract at the end. On the other hand, liquid–liquid extraction of 200 mL of sample using dichloromethane leads only to a 2 mL final extract. As a consequence, carbonyl compounds are 3 times more concentrated in final extracts using Girard's T reagent. The specificity of the method also enables the reduction of number of coelutions, since each sample of freshly distilled spirits contain more than 100 compounds with rather important concentrations.

3.2. Aldehydes

The 21 aldehydes which were identified in Calvados and Cognac are of various types: saturated linear aliphatic ones were found, with 6 (hexanal) to 16 (hexadecanal) carbon atoms, as well as unsaturated ones (pent-2-enal, oct-2-enal and tridec-2-enal). No carbonyl compound containing less than 6 carbon atoms was found because of their coelution with the solvent (diethyl ether) and with the remaining toluene. These low molecular weight compounds like ethanal, butanal and 3- and 2-methylbutanal are certainly present in the diethyl ether extracts, but they have usually already been studied in alcoholic beverages.

Furfural is also a well-known volatile compound of spirits, which was identified in all samples. Mainly produced from the dehydration of pentoses, its presence can be linked to that of 5-methylfurfural which results from the transformation of rhamnose (Mangas et al., 1997). 5-Methylfurfural was detected in both of the extracts of Cognac, but in only one sample of Calvados, in which it presented a very small peak (see Fig. 4).

2-Thiophenecarboxaldehyde was detected in three samples and was quantified by GC/SCD. The presence of 5-methyl-2 thiophenecarboxaldehyde was detected in one sample of Cognac and in one sample of Calvados. *p*-Anisaldehyde was only identified in the Calvados 2 sample, and can be linked to anisyl methyl ketone, which was detected only in the same sample. Three terpenic or norisoprenoidic aldehydes were characterized, and except for *β*-cyclocitral they were only identified in Cognac. 3-Methylthiopropanal (methional), quantified by GC/SCD, could not be found after reaction with Girard's T reagent. This key odorant compound is rather polar, and one can assume therefore that an extraction using toluene does not permit the recovery of a great enough amount. Its presence was verified by preparative GC in a previous study (Ledauphin et al., 2004).

3.3. Ketones

Some terpenoic or norisoprenoidic ketones (4-oxoisophorone and *β*-ionone) also seem to be specific of Cognac. They are in general powerful odorant compounds, and they give their specificity to the Cognac global aroma. A sensory detection limit of 25 $\mu g\,L^{-1}$ was calculated for the 4-oxoisophorone in model port wine solutions (Rogerson et al., 2001) and as a consequence it can contribute to the aroma of Cognac 2 sample where it was identified. *β*-Damascenone, due to its high concentrations in spirits, was identified in all samples, and is already considered as a key odorant of freshly distilled Cognac (Ferrari et al., 2004) and Calvados (Ledauphin et al., 2003). To our knowledge the more saturated *β*-damascone, which was detected in 3 of the 4 samples, has not been identified in spirits previously. Ketones characterized in both spirits are mainly methyl or ethyl ketones. Of interest are heptan-2-one, octan-2-one, nonan-2-one, undecan-2-one and tridecan-2-one, which present very clear chromatographic peaks for all samples. These

Table 4
Results of identification of carbonyl compounds in Cognac and Calvados by GC/MS using Girard's T reagent

Entry	Carbonyl compound	Ret. index	Occurrence in samples (× if detected)			
			Cognac 1	Cognac 2	Calvados 1	Calvados 2
Aldehydes						
1′	Hexanal[a]	1064		×	×	
2′	Pent-2-enal	1073				×
3′	Heptanal	1176			×	×
4′	3-Ethoxypropanal[a]	1185				×
5′	Octanal	1282	×	×	×	×
6′	Nonanal	1387	×	×	×	×
7′	Oct-2-enal	1382		×	×	
8′	2,6,6-Trimethylcyclohex-2-en-1-carboxaldehyde	1420		×		
9′	Furfural[a]	1462	×	×	×	×
10′	Decanal	1501	×	×	×	×
11′	Benzaldehyde[a]	1513	×	×	×	×
12′	5-Methylfurfural	1513	×	×	×	
13′	*β*-Cyclocitral	1606	×	×	×	×
14′	2-Phenylethanal	1631				×
15′	2-Thiophenecarboxaldehyde	1684	×	×		×
16′	5-Methyl-2-thiophenecarboxaldehyde	1767	×			×
17′	Tridec-2-enal	1868		×	×	
18′	3-(2,6,6-Trimethylcyclohexen-1-yl)-prop-2-enal	1920	×			
19′	*p*-Anisaldehyde	1998				×
20′	Pentadecanal	2024			×	
21′	Hexadecanal	2132			×	
Ketones						
22′	Pentan-2-one	< 1000	×	×		
23′	Hexan-3-one	1023			×	
24′	4-Methylpentan-2-one	1059		×		×
25′	Hexan-2-one	1060			×	×
26′	3-Methylpentan-2-one	1067				×
27′	Heptan-3-one	1141	×			×
28′	5-Methylhexan-2-one	1142				×
29′	Heptan-2-one	1173	×	×	×	×
30′	Octan-3-one	1210			×	
31′	Octan-2-one	1269		×	×	×
32′	Oct-1-en-3-one	1277				×
33′	6-Methylhept-5-en-2-one	1327	×	×	×	×
34′	Nonan-2-one	1382	×	×	×	×
35′	Decan-2-one	1493				×
36′	2-Acetylfurane[a]	1500	×	×	×	×
37′	Dihydro-2-methyl-3(2H)-thiophenone	1518	×			
38′	Undecan-2-one	1598	×	×	×	×
39′	Acetophenone	1628	×		×	
40′	2,2,6-Trimethylcyclohex-2-en-1,4-dione (4-oxoisophorone)	1668		×		
41′	Dodecan-2-one	1704	×			
42′	*β*-Ionone	1739	×			
43′	Tridecan-2-one	1805	×			×
44′	*β*-Damascone	1797	×	×		×
45′	*β*-Damascenone[a]	1798	×	×	×	×
46′	Pentadecan-2-one	2019	×		×	
47′	4-(*p*-Methoxyphenyl)-2-butanone	2134				×
48′	(Anisyl methyl ketone)					
49′	Heptadecan-2-one	2231	×		×	
	Total carbonyl compounds identified		25	23	25	29

[a] Compounds already identified in dichloromethane extracts of freshly distilled Calvados and Cognac (Ledauphin et al., 2004).

compounds were already identified in Cognac, and the authors found that their number increases rapidly during the aging process (Watts et al., 2003). Presence of hexan-3-one, heptan-3-one and octan-3-one is more marginal but they were almost detected in one sample. Although recovery of unsaturated carbonyl compounds is rather difficult using Girard's T reagent, some of them could be identified in extracts.

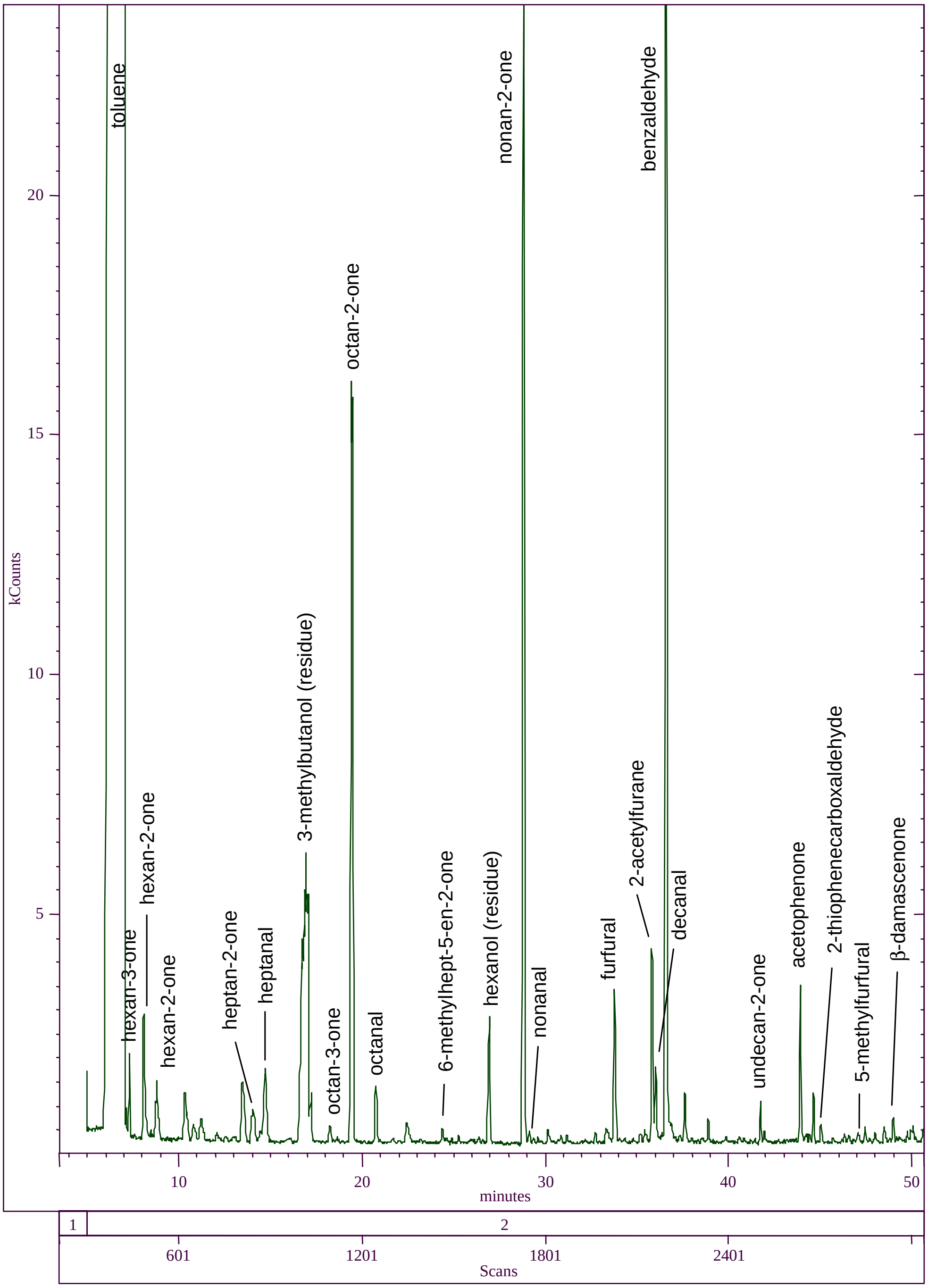

Fig. 4. Chromatogram of Calvados 1 sample Girard's T reagent extract obtained by GC/MS.

Only traces of oct-1-en-3-one were detected in Calvados 2, but the 6-methylhept-5-en-2-one could be clearly identified in all samples. The 2-acetylfurane was also identified in all samples, but the dihydro-3-methyl-3(2H)-thiophenone was only detected in Cognac 1 sample. This sulphur compound was not quantified using GC/SCD, but it was already characterized in samples of freshly distilled Calvados (Ledauphin et al., 2003).

3.4. Sulphur compounds

Sulphur compounds were identified by a comparison of retention times between chromatograms of standard solutions and those of extracts of Calvados and Cognac. The results of identification and quantification are presented in Table 3, and the chromatogram of an extract of Cognac is presented in Fig. 5. For a few compounds already characterized in previous studies,

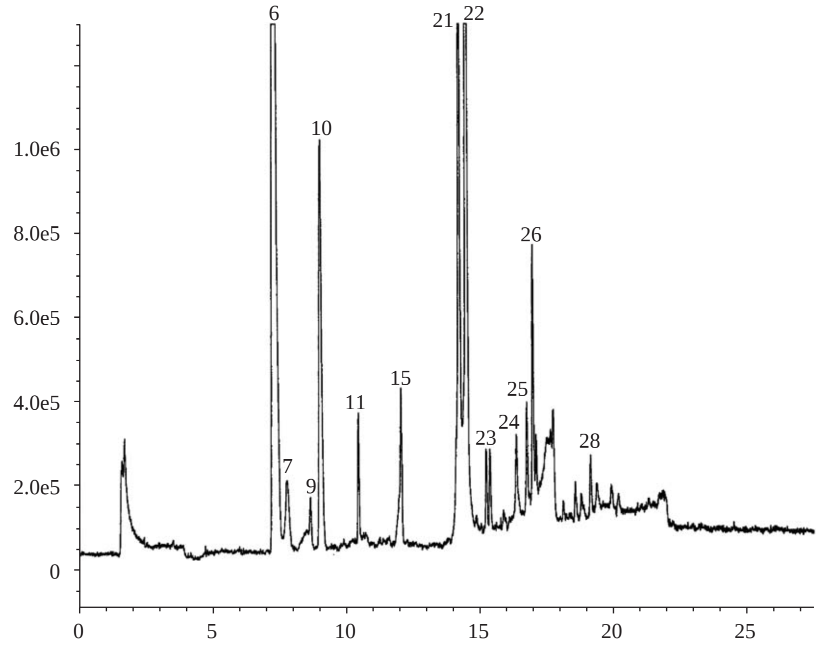

Fig. 5. Chromatogram of Cognac 1 sample obtained by GC/SCD (compounds labelled in Table 3).

concentrations found in Calvados and Cognac are compared to odour detection thresholds determined in wine or water. Global study of sulphur compounds seems to reveal that the level of sulphur compounds in Cognac is five to six times more than in Calvados. Results show that many sulphides such as dimethyl, diethyl, dipropyl and diethyl disulphide are non-existent in these two spirits.

Dimethyl sulphide was characterized in numerous wines, but its concentrations seems to be related to aging (Ferreira et al., 2003) which explains why it could not be found in freshly distilled Cognac or Calvados. Dibutyl sulphide is present in rather high concentrations (between 21 and 97 $\mu g\,L^{-1}$ of PA), whereas ethyl propyl sulphide seems to be specific to Cognac in contrast to Calvados, where only traces are recorded. Relatively high concentrations of dimethyl disulphide are detected (up to $0.5\,mg\,L^{-1}$ of PA) and the other disulphides, except ethyl propyl disulphide, do not seem to be characteristic of these products.

The aromatic impact of dimethyl disulphide in freshly distilled Calvados and Cognac should be rather important because their concentrations, for the most part, reached the odour detection threshold (especially in Cognac 1 and Calvados 1). This compound may be issued from methionine metabolization by lactic acid bacteria in wine (Pripis-Nicolau et al., 2004) or cider. Although concentrations of 4 $\mu g\,L^{-1}$ of PA were present in Calvados, diethyl disulphide was not detected in Cognac.

Thiols, which are responsible for powerful unpleasant sulphurous doors, are often identified in wine, but concentrations of this kind of compound in both Cognac and Calvados are very low. Moreover, they were not detected in Calvados, and only one sample of Cognac (Cognac 1) was found to contain a small amount of 2-methylpropanethiol and butanethiol. Nevertheless, the 17 $\mu g\,l^{-1}$ of PA of 1-butanethiol reported in Cognac 1 should indicate that it may participate to the global aroma of this sample.

Concentrations of 3- and 2-thiophenecarboxaldehyde were carefully examined in samples because these compounds are likely to be potent odorants of Cognac (Ferrari et al., 2004). 2-Thiophenecarboxaldehyde was already identified using Girard's T reagent. The GC/MS peak of that compound must also include its 3 regio-isomer whose mass spectrum is very similar. Both compounds present higher amounts in Cognac. Moreover, it is interesting to note that 3-thiophenecarboxaldehyde is more concentrated than 2-thiophenecarboxaldehyde in Calvados whereas the opposite is observed in Cognac: the ratio of concentrations (3-thiophenecarboxaldehyde/2-thiophenecarboxaldehyde) is between 13 and 15 for Calvados and between 0.2 and 0.5 for Cognac. 3-Methylthiopropanol (methionol), 3-methylthiopropanal (methional) and 3-methylthiopropyl acetate (methionyl acetate) are probably derived from methionine degradation during fermentation. Methionol was presenting a tailing peak in GC and could not be clearly quantified, but it seems to be present in all samples. Low amounts of methional were recorded (up to 39 $\mu g\,L^{-1}$ of PA) but given its extremely low perception threshold it can already be considered as a major key odorant of Calvados (Ledauphin et al.,

2003) and Cognac (Ferrari et al., 2004). The concentration of its derivative methionyl acetate seems to be linked with that of methional via methionol. Indeed, methional was not detected in Cognac 2 and level of methionyl acetate was the lowest in that sample. 4-Methylthiobutanol seemed to be present in only one sample of Cognac. 91 $\mu g\,L^{-1}$ of PA of this compound was quantified in Cognac 2 sample but it was not detectable in other samples. 2-Methylthioethanol was detected in all samples, except in the Cognac 2 sample. Very low amounts of thiophenol were recorded in Cognac, though its concentration seems to be higher in Calvados. Finally benzothiazole, with concentrations between 8 and 20 $\mu g\,L^{-1}$ PA, cannot be considered as characteristic of Cognac or Calvados.

4. Conclusion

Forty-eight carbonyl compounds were identified in freshly distilled Cognac and Calvados. This study shows that the use of Girard's T reagent is suitable for the identification of carbonyl compounds in spirits. However, extraction rate of polar aldehydes and ketones is rather low. This can be compensated for by the efficiency of concentration of the final extracts (up to 300 μL for 100 mL of sample). This method is very specific to carbonyl compounds, and led to the identification of numerous aliphatic aldehydes and ketones. Moreover, a few unsaturated ones, such as oct-1-en-3-one, alkenals and 6-methylhept-5-en-2-one were identified. These compounds are generally powerful odorants and their presence could be directly verified using Girard's T reagent supported on silica-gel. Many terpenic or norisoprenoic compounds, except β-damascenone present in all samples, seem to be specific to Cognac rather than Calvados. Some sulphur carbonyl compounds could also be identified using such a method. Dihydro-2-methyl-3(2H)-thiophenone, 5-methylthiophenecarboxaldehyde and 2-thiophenecarboxaldehyde could be detected in a few samples.

With regard to the above results the methodology was accurate enough to identify and to characterize specifically many of the carbonyl compounds that have never to our knowledge been described in freshly distilled spirits. Nevertheless, some quantitative aspects remain undetermined, such as the comparison of concentration level and olfactive impact for some carbonyl markers (like oct-1-en-3-one, 6-methylhept-5-en-2-one, β-damascenone or 4-oxoisophorone). That is why a new and different method, aimed at quantifying all the carbonyl compounds, is currently being studied in order to evaluate the real olfactive impact of these ones in Calvados and Cognac.

In this study, 28 sulphur compounds were quantified by GC/SCD. It shows that the predominant form of thiophenecarboxaldehyde in Cognac was the 2-regioisomer, whereas Calvados contains a higher content in 3thiophenecarboxaldehyde. Moreover, a larger amount of these two compounds was detected in Cognac. The total concentration of the sulphur compounds quantified in Cognac was 5–6 folds more than that in Calvados. In these, the most important concentrations were recorded for thiophenecarboxaldehydes and dimethyl disulphide. Given its low detection threshold, dimethyl disulphide clearly contributes to the global aroma of Calvados and Cognac. A potato-like odour could be detected in both spirits, as methional may have, in some samples, a concentration 100 times higher than its perception threshold. Significant concentrations of ethyl propyl sulphide, ethyl propyl disulphide and dibutyl sulphide were also detected. The presence of diethyl sulphite seems to be specific to Cognac whereas 4-methylthiobutanol was found only in one sample of Cognac.

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