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## Simultaneous supercritical fluid derivatization and extraction of formaldehyde by the Hantzsch reaction

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### Abstract

A study where the Hantzsch reaction is used to produce the chemical derivatization of formaldehyde in a supercritical medium is presented in this paper. Pressure, temperature and other parameters such as static and dynamic extraction time must be optimized to increase the yield of this kinetically controlled reaction. A  $2^{5-1}$  (resolution V) factorial design was used to study the significant parameters affecting the supercritical process in terms of resolution and sensitivity. A subsequent central composite design was employed to find the conditions of maximum response. Ultraviolet–visible spectrophotometry was used as the detection technique. The optimum conditions were used for the determination of formaldehyde in real finger-paints by means of the previous addition of known quantities of this analyte to the paint. Results were compared with those obtained with supercritical fluid extraction and subsequent chemical derivatization and an improvement of sensitivity as well as a reduction of time of analysis, solvent waste and reagents consumption were observed. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** Hantzsch reaction; Factorial design; Derivatization, SFE; Supercritical fluid derivatization and extraction; Extraction methods; Formaldehyde

### 1. Introduction

Formaldehyde is a colorless gas at room temperature; high volatility and reactivity its two main characteristics. Because of this high reactivity and ability to be a chemical intermediate, formaldehyde is considered a toxic and potentially carcinogenic substance, although this fact has not been confirmed in humans [1]. Some recent papers dealing with the determination of formaldehyde in a broad variety of matrices, such as water [2–6], air [7–9], food, biological samples and plastics [10], coatings [11], and cosmetics [12,13], have permitted the knowledge of the interactions between formaldehyde and the corresponding matrices, as well as the development

of test methods. In the case of finger-paints, the complexity of the matrix makes the development of reliable methods for the determination of formaldehyde really difficult. Formaldehyde is broadly used as a preservative in finger-paints and it is expected that an upper limit ( $\sim 0.1\%$ ) could be imposed for these samples in the near future [14]. Therefore, the proposal of a normalized analytical method to get a reliable and reproducible detection and determination of this analyte makes necessary the elaboration of specific methodology. Only a few works can be found where formaldehyde is directly determined, without any previous derivatization reaction [10,11]. In general, derivatization reactions are normally used, such as those of 2,4-dinitrophenylhydrazine [2–6], chromotropic acid [6], 3-Methyl-2-benzothiazolone hydrazone [6], pararosaniline [7,8],

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2-diphenylacetyl-1,3-indandione-1-hydrazone [9], dimedone [15] and lutidine [12,13,16].

One of the most important methods for the determination of formaldehyde is the lutidine method, which uses the Hantzsch reaction to derivatize formaldehyde to 3,5-diacetyl-1,4-dihydro-2,6-dimethylpyridine [3,5-diacetyl-1,4-dihydrolutidine (DADHL)]. This method is relatively simple, quick, sensitive and requires soft conditions being useful to be employed with supercritical fluids. An important application of this method is the determination of formaldehyde in cosmetics [13]. As these products are chemically similar to finger-paints, it should be expected that the Hantzsch reaction could be equally employed in the determination of formaldehyde in such materials. But the extraction of formaldehyde from matrices is one of the important points to be considered. As conventional extraction techniques, such as Soxhlet, yield low recoveries, the application of newer techniques for extraction is advisable.

The interest in supercritical fluid extraction (SFE) has been growing rapidly during the last years. SFE minimizes sample handling, provides fairly clean extracts, expedites sample preparation and reduces the use of environmentally toxic solvents [17]. Examples of SFE applications include *N*-nitrosamines in food [18], semivolatile compounds [19], polychlorinated biphenyls and polycyclic aromatic hydrocarbons from environmental samples [20], phthalates in poly(vinyl chloride) [21] or aromatic amines in finger-paints [22]. The application of SFE to the extraction of formaldehyde, however, has been quite limited.

However, one of the problems of SFE is the use of supercritical CO<sub>2</sub> as the extraction fluid. Because of its low polarity the extraction of polar analytes, such as formaldehyde, is difficult and recoveries are poor. An alternative way to work with supercritical fluids is through chemical derivatization, which permits the decrease of the polarity of polar analytes, the increase of their volatility and solubility in the supercritical fluid and their easy separation from aqueous and solid samples. The simultaneous supercritical fluid derivatization and extraction (SFDE) is not employed in a high number of applications, but some work on organometallics from sediments and soils [23], caffeine in coffee beans [24], phenol in wood soot leachate [25] or alkylbenzenesulfonates in wastewater sludge [26] can be found in the literature.

A formal approach is convenient to study new systems where several factors can be interacting and more information is obtained with few runs by varying several factors at once [27,28]. For this reason, factorial designs are interesting in SFE applications for different samples [22,29–34]. In this case, a 2<sup>5-1</sup> (resolution V) factorial design was used to study the influence of several parameters on derivatization and extraction in terms of resolution and sensitivity. An extra central composite design was also developed to define the response surface as a function of the significant parameters obtained from the previous design.

The aim of the present work is the study of the Hantzsch reaction as a method of derivatization of formaldehyde under supercritical conditions. Moreover, a comparison with SFE and subsequent derivatization is included.

## 2. Experimental

### 2.1. Materials and chemicals

All reagents were analytical grade and obtained from Panreac (Barcelona, Spain) and Normapur (Prolabo, Barcelona, Spain). A formaldehyde standard solution (38%, m/v) was used to prepare all solutions that were standardized iodimetrically.

### 2.2. Supercritical fluid derivatization and extraction

SFDE was performed (off-line mode) using an ISCO Model SFX-220 extraction system (ISCO, Lincoln, NE, USA) consisting of an SFX-220 extractor, a SFX-200 controller and a 100DX-syringe pump. Supercritical grade CO<sub>2</sub> was obtained from Abelló Linde (Valencia, Spain). A 0.20±0.01-g amount of sample was introduced into a stainless steel cartridge (internal volume, 2.5 ml). All the reagents were added directly to the cartridge and a small amount of quartz wool, which helps to minimize the dead volume of the cartridge, was added. The capillary restrictor was coaxially heated and the temperature was programmed to 215°C. In order to trap the extracted derivatization product, the outlet of the restrictor was introduced into a double vial tandem [21] with 5 ml of distilled water in each one.

All extractions were carried out in the static/dynamic mode, with the use of the selected static and dynamic extraction times. The final extract was then diluted to 15 ml.

### 2.3. Design of experiments

A  $2^{5-1}$  fractional factorial design for a commercial paint fortified with  $1.5 \cdot 10^{-5}$  mol of formaldehyde was carried out to distinguish the significant parameters affecting the supercritical process. The initial parameters to be included in the design were pressure, temperature, static and dynamic extraction times and acetylacetone volume. A 100- $\mu$ l volume of an acetic acid–ammonium acetate buffer (pH 6.4) [16] was also added for each experiment. A  $0.60 \pm 0.01$ -g amount of wet paint was fortified with 0.25 ml of a formaldehyde aqueous solution (0.06 M) before the extraction. The results of the initial design were used to plan a subsequent higher order design (central composite), which was performed with the same procedure.

### 2.4. UV–Vis spectrophotometry

Spectra from 190 to 1100 nm and the absorbances at 410 nm of the final extracts with distilled water as reference were considered. The absorbance is associated with the product of the condensation of formaldehyde with ammonia and acetylacetone to form DADHL. UV–Vis detection was carried out with a UV-1603 Spectrophotometer (Shimadzu, Kyoto, Japan).

For the second part of this work, the standard addition method was employed to determine the content of formaldehyde in several finger-paints. The SFDE conditions were those of the optimal point found in the response surface defined by a central composite design.

### 2.5. Supercritical fluid extraction and subsequent derivatization

In order to compare the results with those obtained by the use of supercritical fluid extraction and subsequent derivatization (SFE+D), experiments were run in a similar way. From the optimum results of a previous factorial design, SFE was carried out at 13.8 MPa, 120°C, 15 min of static extraction time,

15 min of dynamic extraction time and 80  $\mu$ l of modifier (methanol). In order to trap the extracted formaldehyde, the outlet of the restrictor was introduced in a double vial tandem with 5 ml of methanol–water (10%, v/v) in each one and coaxially heated at 85°C. The final extract was then diluted to 15 ml. A 1-ml volume of the extract was then mixed with 5 ml of the acetic acid–ammonium acetate buffer (pH 6.4), diluted to 25 ml and heated at 60°C during 10 min [16]. The obtained DADHL was finally extracted with 10 ml of 1-butanol and the absorbance was measured using 1-butanol as a reference.

## 3. Results and discussion

The effect of the different variables affecting the supercritical derivatization and extraction of formaldehyde in finger-paints was studied by a  $2^{5-1}$  fractional factorial design with two levels (low and high) for five factors. This half-fraction of a  $2^5$  design was obtained by substitution of a fifth factor with the highest order interaction between four factors in a complete  $2^4$  factorial design. Then, the design generator could be described as  $E = +ABCD$ ;  $A$ ,  $B$ ,  $C$ ,  $D$  and  $E$  being the five factors. This design requires 16 experiments, performed randomized. It is assumed that only the main factors and second-order interactions between factors are significant for the process [27,28]. An extra experiment was included to have a rough estimation of the responses in the center of the design. Attending to the unknown behavior of the Hantzsch reaction under supercritical conditions, the selected parameters were  $\text{CO}_2$  pressure ( $P$ ), extraction temperature ( $T$ ), static and dynamic extraction time ( $s$  and  $d$ ) and volume of acetylacetone ( $c$ ). The low and high values for each parameter were selected according to the experimental limitations and coded to be  $-1$  and  $+1$  from the center of the design (0 for each parameter). With this transformation all parameters are independent of the units. The evaluated response parameters were resolution ( $R_s$ ) and sensitivity ( $S$ ). The former was chosen to avoid the overlapping of the peak corresponding to DADHL and the co-extracted substances in the UV–Vis spectrum, as can be seen in Fig. 1. Resolution was calculated as a ratio between several terms. In the numerator: the absorbance at 410 nm,

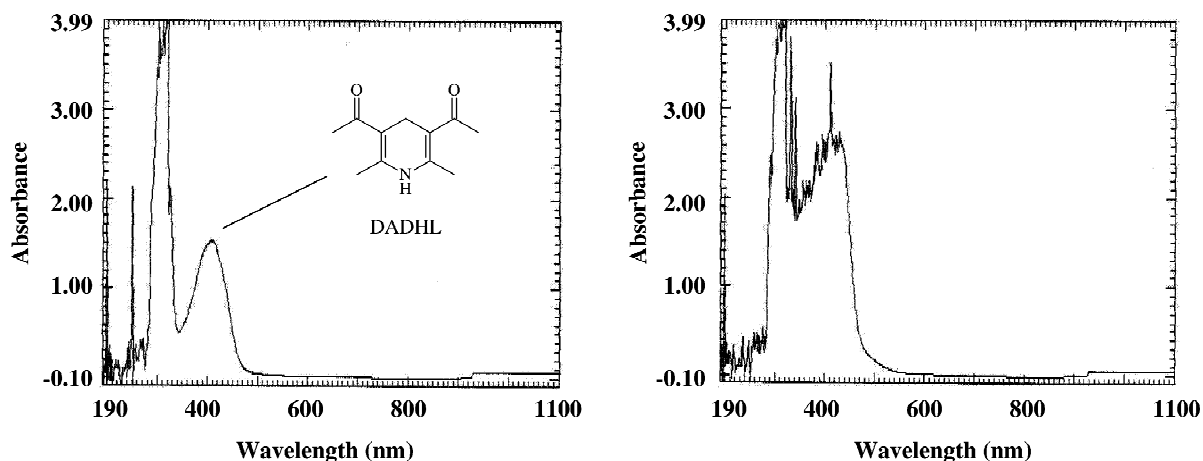


Fig. 1. UV-Vis spectra of maximum resolution (left) and maximum sensitivity (right) from the  $2^{5-1}$  factorial design.

the difference between the absorbance at 410 nm and the absorbance in the valley between 320 and 410 nm and the ratio between minimum and maximum absorbances around 410 nm. In the denominator: the absorbance at 320 nm and the absorbance in the valley between 320 and 410 nm. The second parameter was selected because of its interest as an analytical variable to give the highest signal per mol of formaldehyde present in the sample. Therefore, sensitivity was defined as absorbance at 410 nm.

Table 1 lists the values of each factor and the results for each response in the different experiments.

In order to stabilize the variance of results, an appropriate potential transformation ( $Y=y^\lambda$ ) of either response ( $y=R$  or  $y=S$ ) was carried out before the analysis of results. A suitable transformation of response is recommended when big differences between the values of response are found [28]. The best transformation is achieved when the sum of squares of residuals is the lowest as a function of the

Table 1  
List of experiments in the  $2^{5-1}$  factorial design with resolution V

Experiment	$P$ (MPa)	$T$ (°C)	$s$ (min)	$d$ (min)	$c$ ( $\mu$ l)	Resolution	Sensitivity
1	13.8	50	2	2	100	0.00025	0.057
2	55.1	50	2	2	10	0.33333	0.094
3	13.8	120	2	2	10	0.02857	0.017
4	55.1	120	2	2	100	0.20362	1.561
5	13.8	50	15	2	10	0.00027	0.010
6	55.1	50	15	2	100	0.52632	0.673
7	13.8	120	15	2	100	0.00026	0.028
8	55.1	120	15	2	10	0.52632	1.144
9	13.8	50	2	15	10	0.42568	0.142
10	55.1	50	2	15	100	0.60150	0.399
11	13.8	120	2	15	100	0.06098	0.767
12	55.1	120	2	15	10	0.74165	1.086
13	13.8	50	15	15	100	0.08780	0.252
14	55.1	50	15	15	10	0.40000	0.379
15	13.8	120	15	15	10	0.00026	0.028
16	55.1	120	15	15	100	0.28091	3.105
Central	34.5	85	8.5	8.5	55	0.75000	1.836

exponent  $\lambda$ . The optimum transformation for resolution ( $Y=R^\lambda$ ) was obtained for  $\lambda$  between  $-0.85$  and  $1.65$ . The significant factors and interactions were identified by using normal probabilistic plots, where all negligible factors and interactions are expected to be located along a straight line and used to estimate the variance of the design. By contrast, points that fall well off the line would suggest the existence of a significant influence. These plots were employed because the degrees of freedom of the design are not enough to calculate the error. Fig. 2 shows the normal probabilistic plot for resolution, where  $\lambda=1.6$  was selected because of the good correlation coefficient of the straight line. This figure shows two main factors, pressure and dynamic extraction time, which are significant with positive effects. Moreover, two negative interactions are present; between temperature and volume of acetylacetone and between static and dynamic extraction times. The effects of  $P$  and  $d$  can be explained attending to the solubility and sweeping of DADHL from the supercritical fluid extractor. However, the explanation of the two negative interactions

is not immediate. The main goal is the reduction of the interfering reactions and improvement of the production of DADHL. According to this,  $T$  with  $c$  as well as  $s$  with  $d$  have opposite effects. The  $T^*c$  interaction can be justified considering that  $T$  and  $c$  increase the velocity of the Hantzsch reaction as well as the possibilities of finding lateral reactions. Therefore, from Fig. 3, increasing  $T$  and reducing  $c$  seems to be better than to increase the response. On the other hand, the  $s^*d$  interaction can be explained considering that the bigger improvement in  $R$  is obtained when  $s$  is short and  $d$  is long. In this manner, the development of interfering reactions is reduced and the sweeping of the DADHL formed is enhanced.

A similar analysis was carried out for sensitivity resulting in a potential transformation  $Y=S^{1.1}$  according to the same considerations. From the normal probabilistic plot, shown in Fig. 4, pressure, temperature and volume of acetylacetone as well as the interaction between pressure and temperature appear to be significant with a positive effect. These results can be explained in terms of the assumption that the

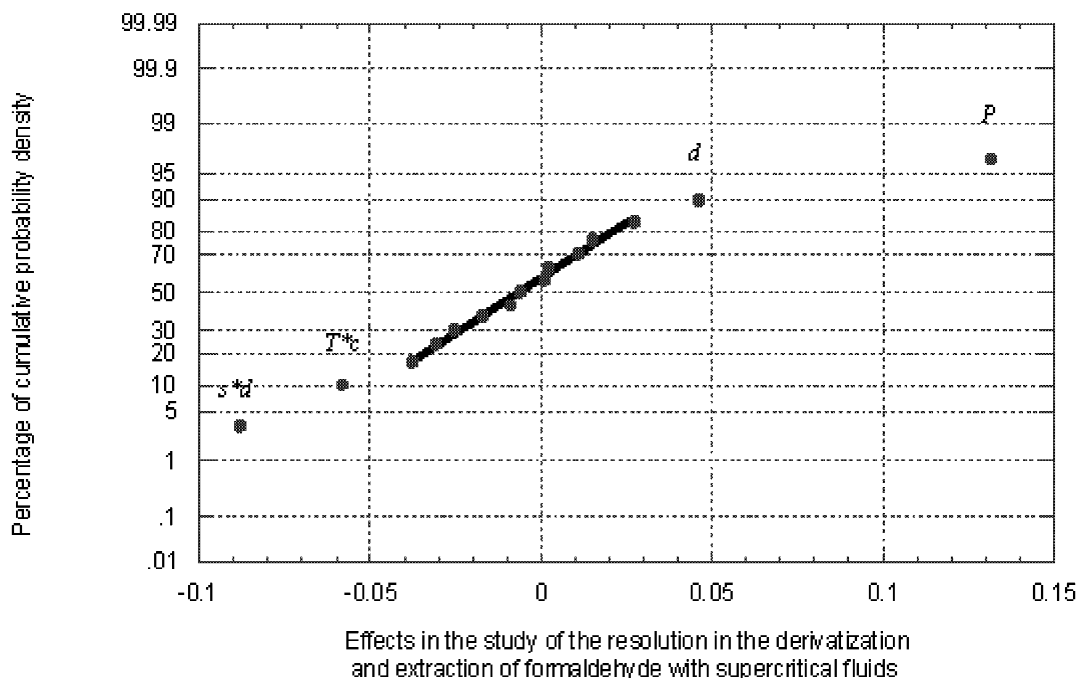


Fig. 2. Normal probabilistic plot of cumulative probability density function vs. calculated effects for  $Y=R^{1.6}$ .

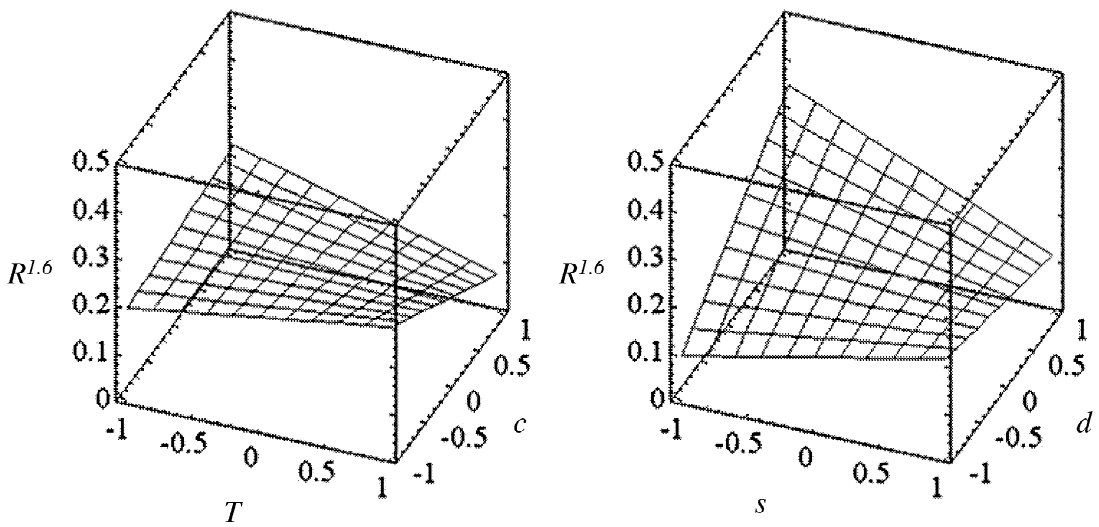


Fig. 3. Significant interaction for resolution from the  $2^{5-1}$  factorial design.

three main factors and the significant interaction can be directly associated with an increase in the velocity of the Hantzsch reaction. Moreover,  $P$  can contribute, as in  $R$ , in the polarity of supercritical  $\text{CO}_2$  and so, in the solubility of the DADHL. The positive sign

of the  $P*T$  interaction could be related with a synergetic effect produced by a simultaneous increase of the probability of efficient collisions between the reagents and their thermal agitation.

Further optimization is not easy to plan in terms of

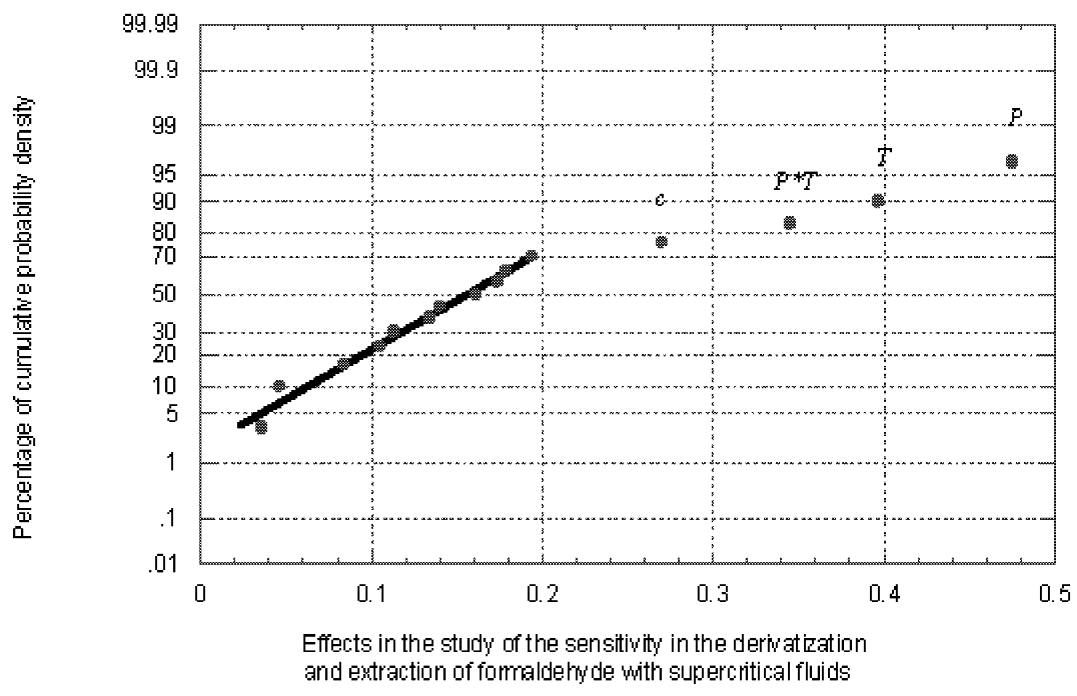


Fig. 4. Normal probabilistic plot of cumulative probability density function vs. calculated effects for  $Y=S^{1.1}$ .

$R$  because two second-order interactions are present and only the significant main factor  $d$  is included in one of them ( $s*d$ ). By contrast,  $S$  presents only one interaction ( $P*T$ ) directly related with two significant main factors. Moreover, absorbance at 410 nm defines specifically  $S$  and is included in  $R$ . For this reason, an enhancement in  $S$  results in an improvement in  $R$ , making possible a better comprehension of the supercritical phenomena.

As the increase of volume of acetylacetone could lead to the loss of the supercritical condition of the fluid into the extractor, this parameter as well as those considered non-significant, were maintained in their high levels to study the effect of pressure and temperature in more detail. Therefore, an extra central composite design was carried out for these two factors. This design was constructed by addition of a factorial design  $2^2$  and a star design, with experiments in the centre point and others located at an adequate distance from the center point. This distance was selected to be equal to  $-1.414$  and  $+1.414$  to assure the rotatability condition of the central composite design. Rotatability makes the uncertainty of the design only dependent on the distance to the center of the working range [28]. Furthermore, to obtain a rotatable design with uniform precision, five center points were used [27]. Contraction on the initial levels of pressure and temperature were done to avoid that the expansion in the star of the central composite design falls out the

Table 2  
List of experiments in the central composite design for pressure ( $P$ ) and temperature ( $T$ )

Experiment	$P$ (MPa)	$T$ (°C)	Sensitivity
1	19.8	60	0.134
2	49.1	60	1.015
3	19.8	110	0.134
4	49.1	110	1.168
5	34.5	50	0.557
6	34.5	120	1.299
7	13.8	85	0.035
8	55.1	85	1.083
Central 1	34.5	85	1.061
Central 2	34.5	85	0.971
Central 3	34.5	85	1.277
Central 4	34.5	85	1.055
Central 5	34.5	85	0.905

experimental limits of the extractor. In this manner, estimation of the response surface can be done without instrumental limitations. The required experiments and results are presented in Table 2. The obtained results are shown as a response surface in Fig. 5 where a maximum is found at 45.4 MPa, 105°C, using 15 min static and 15 min dynamic extraction times, 100  $\mu$ l of acetylacetone and 100  $\mu$ l of the acetic acid–ammonium acetate buffer. Therefore, it can be considered that these are the optimum conditions for the supercritical extraction of formaldehyde in finger-paints. Fig. 6 shows that an improvement in resolution and sensitivity was obtained when compared to the best results for each one in the first design.

These optimum experimental conditions were applied to the determination of formaldehyde in real finger-paints by the standard addition method. Table 3 presents the results for several commercial finger-paints. Four aliquots of each sample were fortified with different known quantities of formaldehyde (between 7.5 and  $600 \cdot 10^{-7}$  mol with 0.25 ml of the suitable solutions) and measured in triplicate. Absorbances were then related to the quantities of formaldehyde added. These results were compared with those obtained by derivatization of formaldehyde after the SFE using the standard addition method in the same way. Although no significant differences were observed between the two procedures, the simultaneous supercritical derivatization and extraction permits the reduction of handling and time of analysis, saving of expensive reagents and reduction of solvent waste.

Table 3  
Determination of formaldehyde by standard addition method in several finger-paints<sup>a</sup>

	Formaldehyde in finger-paint ( $\mu$ g/g)	
	SFE+D	SFDE
Finger-paint 1	547 $\pm$ 53	539 $\pm$ 67
Finger-paint 2	764 $\pm$ 39	793 $\pm$ 62
Finger-paint 3	487 $\pm$ 49	441 $\pm$ 38
Finger-paint 4	742 $\pm$ 84	756 $\pm$ 93

<sup>a</sup> SFE+D: Supercritical fluid extraction and subsequent derivatization. SFDE: In situ supercritical fluid derivatization and extraction. Confidence intervals are calculated for 95% probability.

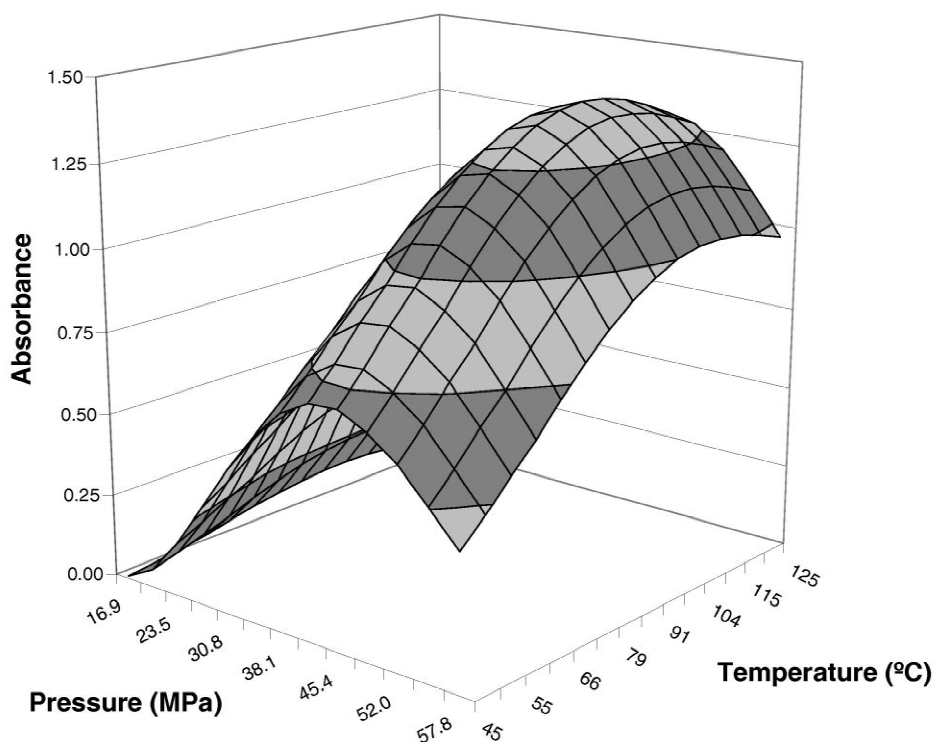


Fig. 5. Response surface for pressure ( $P$ ) and temperature ( $T$ ) from the central composite design.

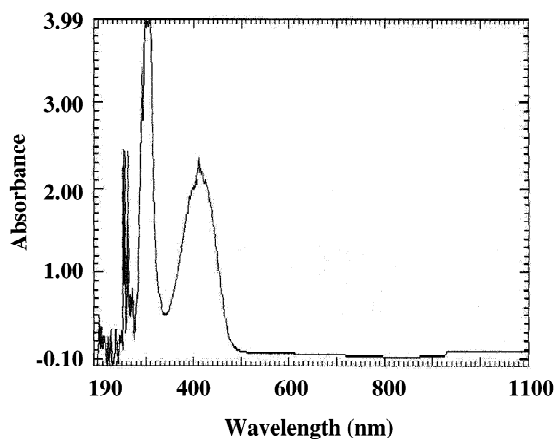


Fig. 6. UV-Vis spectrum of optimum sensitivity from the central composite design.

#### 4. Conclusions

SFDE is a simple and effective method to get a reliable and fast analysis with a reduction in solvent waste and time. The Hantzsch reaction is a selective way of derivatization for formaldehyde from fingerprints in supercritical conditions. Factorial designs can be considered as an effective method to study the influence of several parameters and permit the acquisition of more robust results with a reduced number of experiments when compared to the classical one-to-one parameter approach. The fluidity of finger-paints permits the application of the standard addition method including the variability along the whole procedure for the determination of formaldehyde. Relatively reproducible and reliable results are obtained with independence of the differences between matrices and the possible shortcomings of any step included in the determination.



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