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# DEVELOPMENT OF NEW GAS CHROMATOGRAPHY/MASS SPECTROMETRY PROCEDURE FOR THE DETERMINATION OF HEXAHYDROPHTHALIC ANHYDRIDE IN UNSATURATED POLYESTER RESINS

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**ABSTRACT**

This paper reports the first contribution to the determination of the hexahydrophthalic anhydride in unsaturated polyester resins by automated head space/solid-phase micro extraction and gas chromatography/mass spectrometry analysis. After a preliminary reaction of the acid anhydride with water to form carboxylic acids, a rapid on-sample derivatization using trimethyloxonium tetrafluoroborate was used. A new autosampler platform was proposed in this study by using the Multi Fiber Exchange device. The limits of detection for one mg of unsaturated polyester resin were 2.9 pg for the *cis*-1,2-cyclohexanedicarboxylic acid and 8.0 pg for *trans*-1,2-cyclohexanedicarboxylic acid, whereas the limits of quantification were 14.5 and 29.7 pg, respectively. The equilibrium and kinetics of this substance vs solid-phase microextraction are theoretically evaluated and discussed.

**Keywords:** Trimethyloxonium tetrafluoroborate, Hexahydrophthalic anhydride, unsaturated polyester resins, Gas chromatography, Solid phase microextraction.



## INTRODUCTION

Hexahydrophthalic anhydride (HHPA), both the *cis*- and *trans*- form, is mainly used as intermediate for coating resins, plasticizers, insect repellents and rust inhibitors, and as hardener for epoxy resins. HHPA is preferred over other cyclic anhydrides in casting and coating applications for his higher resistance to yellowing. Especially, unsaturated polyester resins (UPR) are thermosetting resins produced by the reaction of polyesterification between dicarboxylic acids and glycols. The final mixture is obtained by dissolving the resulting resin in an unsaturated and reactive solvent, normally styrene.

This paper reports the first contribution to the simultaneous preparation and determination of the HHPA in UPR by automated head space (HS)/solid-phase microextraction (SPME)/gas chromatography (GC)/mass spectrometry (MS) analysis. After a preliminary reaction of the acid anhydride with water to form carboxylic acids, a rapid derivatization using trimethyloxonium tetrafluoroborate (TMO) was used. On-sample derivatizations followed by HS/SPME and their simultaneous GC/MS analysis has been described for the determination of organic acids in aqueous matrices[1]. These methods employ a sample derivatization technique to convert such polar substances into hydrophobic compounds whose volatility is sufficiently high for a GC determination. Trialkyloxonium salts, also known as Meerwein's salts, are powerful alkylating agents[2-8]. In combination with a hindered base is a convenient reagent for the conversion of carboxylic acids to their methyl esters by O-alkylation[9,10]. The applications of TMO as a methylating agent were implemented in this work. The reaction occurs under mild conditions at room temperature, and it is completed in a few minutes.

In the last 10 years, miniaturization has attracted much attention in analytical chemistry and has driven solvent and sample savings, sample enrichment, rapid sample preparation, and easier automation. Amounts of organic solvents used in analytical laboratories has been reduced by applying solventless extraction, extraction using other types of solvent, assisted solvent extraction and miniaturized analytical systems. The principles of green chemistry are applied to not only chemical engineering and synthesis, but also increasingly analytical chemistry[11-14]. The SPME sampling technique, introduced by Pawliszyn at the beginning of the 1990s, uses a fiber core coated with sorbing materials, assembled inside a syringe needle[15]. SPME integrates sampling, extraction, concentration and sample introduction into a single step and the extraction requires no polluting organic solvent[16-19]. Within analytical chemistry, the SPME analysis is considered one of major ideas that shaped 20th-century analytical chemistry[20].
The object of this work is to obtain assays in automated very short time windows, with consequently more sensitivity power and high discrimination, by structurally informative MS fragmentation pattern, than other techniques used in industrial chemistry laboratories for routine analysis. We proposed a new off-line platform, called SPME Multi Off-Line Sampler, coupled to MultiFiber Exchange (MFX) installed on a *xyz* autosampler. This increased versatility of MFX allows on-sample SPME derivatization approaches in automated mode, therefore Authors are interested to methyl ester of carboxylic group where selectivity and sensitivity of the analytical method may be greatly improved by use of GC/MS.

## EXPERIMENTAL

### Materials and Methods

Cyclopentanecarboxylic acid (CAS No. 3400-45-1, Cat. No. C112003, Aldrich), *cis*- (CAS No. 85-42-7, Cat. No. 123463, Aldrich) and *trans*-HHPA (CAS No. 14166-21-3, Cat. No. 148296, Aldrich), *cis*-1,2-cyclohexanedicarboxylic acid (*cis*-CHDA), (CAS No. 610-09-3, Cat. No. S443824, Aldrich), *trans*-1,2-cyclohexanedicarboxylic acid (*trans*-CHDA) (CAS No. 2305-32-0, Cat. No. 147516, Aldrich), trimethyloxonium tetrafluoroborate (TMO) (CAS No. 420-37-1, Cat. No. 281077, Aldrich), 85 µm polyacrylate (PA) fiber (Cat. No. 57305, Supelco), 85 µm PA SPME Fast Fit Assemblies fiber (Cat. No. FFA57294-U, Supelco).

### General Procedure

Two mL of diluited UPR (Fig.-1) were transferred into a 20 mL autosampler vial with a magnetic stirring bar and mixed with 40 µL of the internal standard (IS) cyclopentanecarboxylic acid, water solution (250 µg/mL). For calibration curve, *cis*- and *trans*-HHPA methanolic solution (1 mg/mL) were added in UPR. To convert the *cis*-CHDA and *trans*-CHDA into their methyl esters, derivatization with TMO was performed at room temperature in two steps. While stirring about 20 mg of $Na_2CO_3$ were added and within 4 minutes approximately 30 mg of solid TMO were added in two aliquots. After 1 minute the solution was neutralized with about 15 mg of $NaHCO_3$. This procedure was repeated again. Finally, for HS/SPME, sodium chloride (0.5 g) was added and the vials were processed by on- or off-line extraction.

### Detection Method

On-line SPME procedure was achieved using a new Flex *xyz* autosampler (EST Analytical). The syringe injector of the SPME unit, equipped with 85 µm PA fiber was used for the extraction procedure. For HS-SPME absorption, a pulsed agitation (on for 2 s at 500 rpm and off for 4 s, 50 °C) was carried out for incubation, before automatically introducing the fiber into the vial in the same conditions. After the absorption the SPME fiber was introduced into the GC injector port by autosampler.
Simultaneously, the On-line SPME procedure was compared to the SPME Multi Off-line technique. This apparatus (Chromline, Prato, Italy) is designed to be used with SPME FFA fibers. The holder works as a support to expose the SPME fiber, in this case FFA 85 µm PA fiber, into the vial, place on plate of the 32-position magnetic stirrer (Chromline). After the exposure the FFA is removed from the Multi Off-Line Sampler (Fig.-2) and place into the 45-position MFX, installed on Flex autosampler for the desorption into the GC instrument equipped with Merlin Microseal System (Cat. No.24817-U, Sigma-Aldrich). The MFX allowed the automated exchange of SPME FFA fibers.

Analysis was performed with a Varian CP-3800 GC equipped with electronic flow control and a Saturn 2200 Ion Trap-MS (Varian Inc.) detector. A fused silica methyl-deactivated capillary column (internal diameter, 10 m × 0.25 mm) was used as a guard column connected to a VF-5 ms (internal diameter, 30 m, 0.25 mm and film thickness, 0.25 μm) analytical column (Cat. No. CP9013, J&W GC Columns, Agilent Technologies). The initial column temperature was set to 50 °C for 1 min and then increased at 10 °C/min to 240 °C (total run time 20.00 min). For desorbing the analytes, the SPME fiber was introduced into the 1079 Varian GC injector port (10:1 split mode) and maintained at 300 °C, for 4 min. The MS was operated in electron ionization (EI) mode. Helium (99.999%) at a flow rate of 1.2 mL/min was used as carrier gas. We operated for all the other compounds in EI mode, full-scan, using as quantification ion the base peak from the 70 eV EI spectra as follows: methyl esters of *cis*- and *trans*-CHDA, m/z =81, and IS, m/z= 87.

Calibration samples were prepared and analyzed to obtain a calibration curve. Six calibration standard samples were obtained, and five analyses were performed for each one. Least-squares linear regression analysis of response factor plot was used to estimate slopes (m) and intercepts (b) of calibration lines y = mx + b, where y is the ratio between the chromatographic area of the analyte and the relative IS, and x the concentration of the analyte (pg/mg of UPR). The limit of detection (LOD) of the assay was calculated according to the expression-

$$LOD = (3SE_b + b)/m$$

Where, $SE_b$ is the standard error of the intercept [21]. Lower limit of quantification (LOQ) is then estimated in the same way using $10SE_b$, which corresponds to 3.3 LOD. The precision of the assay (as a coefficient of variation, CV%) was estimated both as within session and as inter-session repeatability. Within session accuracy was evaluated by the recoveries (reported as the percentage ratio between the measured and the nominal concentrations in water solution) at all concentrations used for the calibration plot. Values of the accuracy were compared with the requirements of US Food and Drug Administration for analytical method validation.

## RESULTS AND DISCUSSION

Sampling of resins by HS-SPME sampling and following GC/MS (Fig.-3 and Fig.-4) analysis has aroused interest in the authors of this work and has been investigated as a possible alternative to conventional methods. The aim of this paper is to provide a simple, fast, sensitive, and organic-solvent free innovative procedure for analysis of HHPA in UPR. So, to achieve successful method, two fundamental requisites were satisfied by the Authors.

### Verify the suitability to HS-SPME technique on the organic acid derivatives

The first objective was to develop the derivatization conditions onto HS-SPME technologies to obtain compounds which are stable under a variety of conditions and easily amenable to sampling, to GC separation, and to MS identification. Since the performance of this technique for HHPA determination in UPR previous reaction with water and methylation of related organic acids with TMO has not been reported, the equilibrium and kinetics of *cis*- and *trans*-CHDA-methyl ester, with regards to SPME have been discussed on a theoretical basis or experimentally determined. The PA absorptive liquid coating was chosen for the sampling of a very complex matrix such as UPR because of the lack of competition between the analytes, and therefore cyclopentanecarboxylic acid could be used as the IS instead of specific isotope-labelled compounds. The HS-SPME techniques were described in a previous work [22] by examining a three-phase system in which a liquid polymeric coating, a headspace and an aqueous solution were involved. The mass (n) of analytes absorbed by a coating after the equilibrium has been reached is related to the overall equilibrium of analytes in a three phase system-

$$n = (C_0V_1V_2K_1K_2)/(K_1K_2V_1 + K_2V_3 + V_2)$$

Where, $K_1$ is the SPME coating/HS partition coefficient, $K_2$ is the HS/aqueous matrix partition coefficient, $C_0$ is the initial concentration of the analyte in the aqueous solution, and $V_1$, $V_2$ and $V_3$ are the

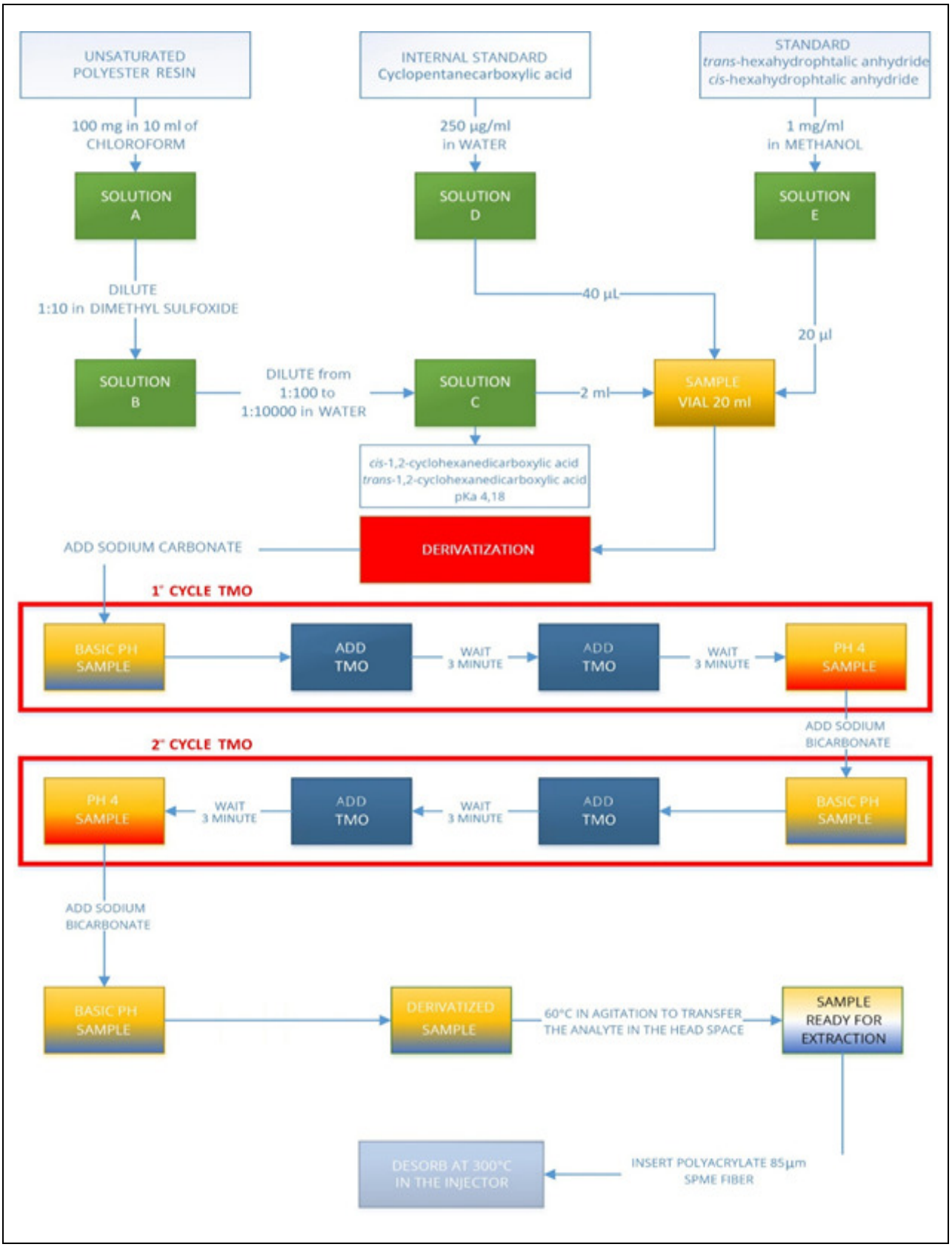



Fig.-1: On-sample derivatization by TMO procedure.

volumes of the coating, the aqueous solution, and the HS, respectively. Since K values of the analytes (where $K = K_1 \times K_2$) are often very close to the octanol–water partition coefficient ($K_{ow}$), and $K_2 = K_H/RT$, where $K_H$ is Henry's constant ($C_0$, concentration gas phase/$C_0$, concentration liquid phase). It derives that the equilibrium is controlled by $K_{ow}$ and $K_H$ values. The values $K_{ow}$ and $K_H$ can be found in the literature, and in this way it is possible to know in advance whether or not the HS-SPME method offers some advantages.

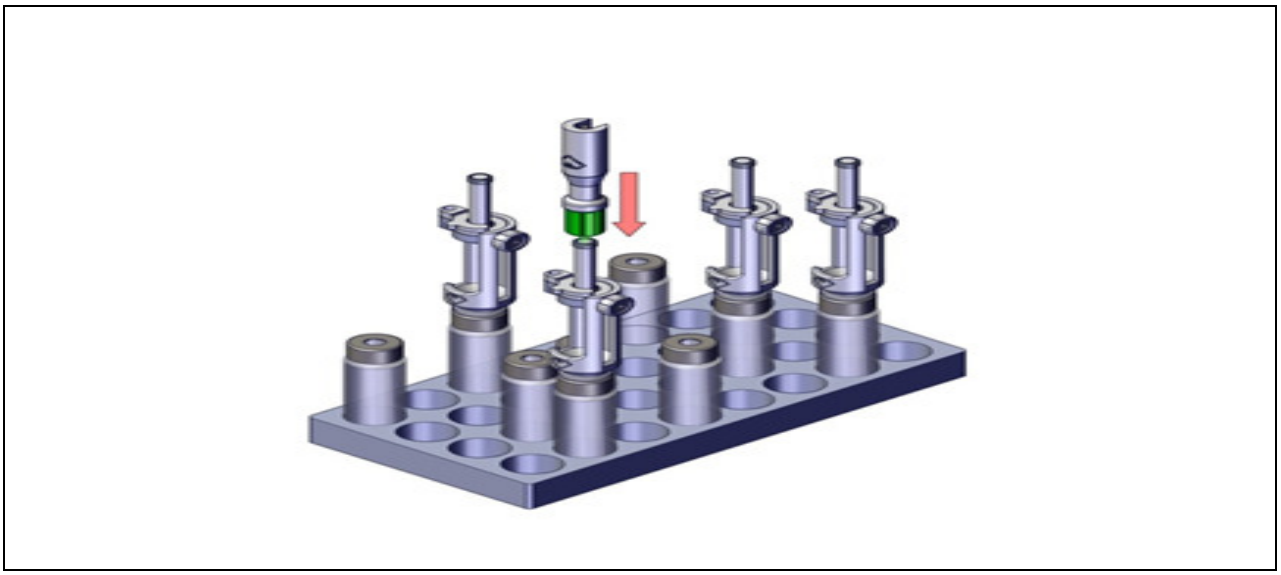


Fig.-2: SPME 32-position Multi Off-Line Sampler.

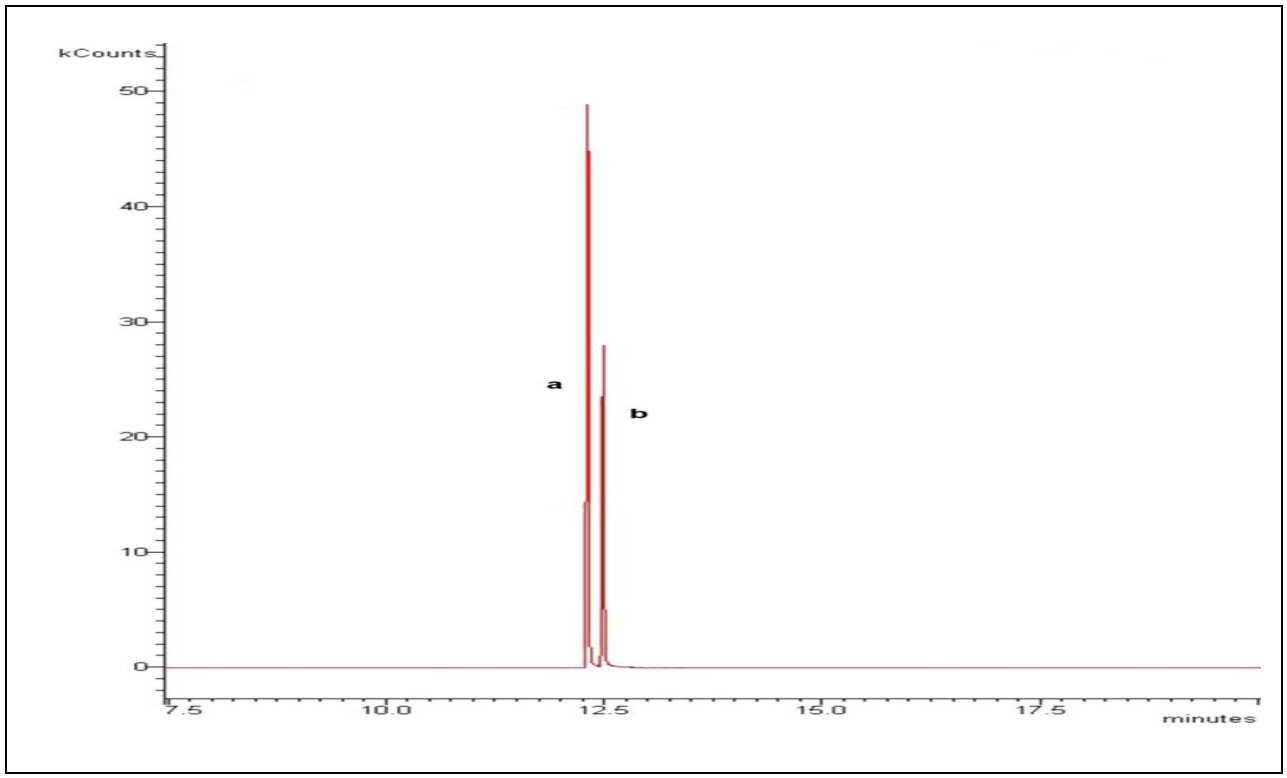



Fig.-3: GC chromatogram of *trans*-(a) and *cis*-(b) CHDA-methyl esters.

An effective use of the theory minimizes the number of experiments to be performed; however, the assumption of ideal conditions required by the mathematical modelling require verification. Therefore, the constant of distribution estimated from physicochemical tables or by using the structural unit contribution method can anticipate trends in SPME analysis. Furthermore, Performs Automated Reasoning in Chemistry is a physicochemical calculator that uses computational algorithms based on the fundamental chemical structure theory to estimate a wide variety of reactivity parameters strictly from molecular structures.[23]

The constant of Henry of the CHDA-methyl ester derivative was 0.549 atm $cm^3$/mol, which was in agreement with that reported by Pacenti et al [24], and indicated that HS-SPME is efficient for compounds with the $K_H$ higher than 0.17 atm $cm^3$/mol. Table-1 illustrated the physicochemical constants of the CHDA-methyl esters to anticipate trends in sampling extraction.

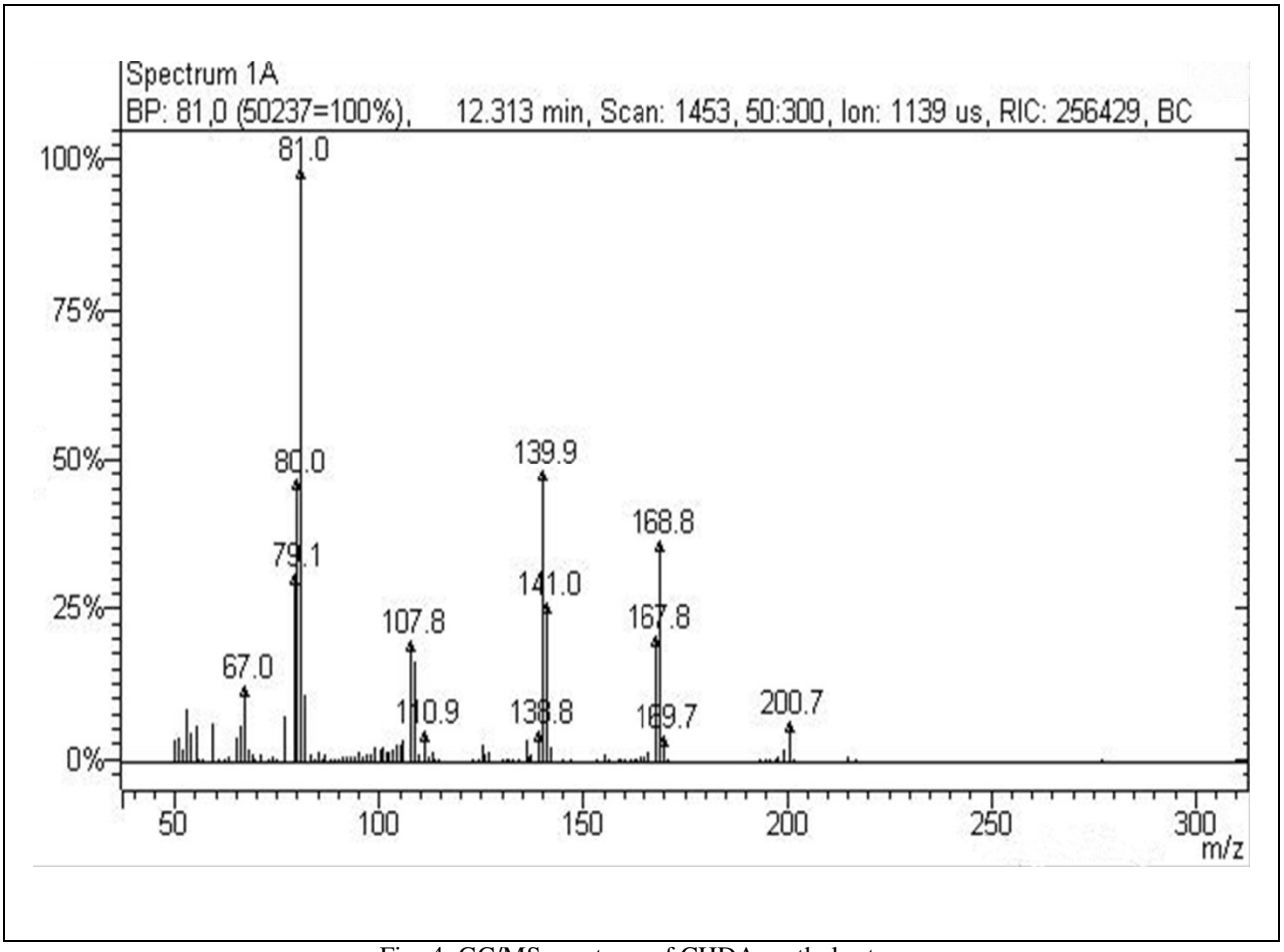



Fig.-4: GC/MS spectrum of CHDA-methyl ester.

**Automation of the Procedure**

In the last 10 years, miniaturization has attracted much attention in analytical chemistry and has driven solvent and sample savings, sample enrichment, rapid sample preparation, and easier automation, resulting in the proliferation of *xyz* autosamplers. Sample preparation remains one of the more time-consuming and error-prone aspects of analytical chemistry. New sample preparation techniques are being increasingly introduced because of the considerable need for information management, the automation of sample preparation, and the integration of data management into the analytical process. Modern autosamplers and workstations possess a range of capabilities, in addition to simple liquid injection, that allow the automation of sample preparation steps traditionally performed manually. Furthermore, the

flexibility of the *xyz* robotic autosampler has been useful to setup and integrate all sampling management processes and software implementation of the Flex GC autosampler. A connection with the Laboratory Information Management System (Bika Lab System) allows a user-programmable suite; therefore, customized processing steps could be easily created by the analyst. The new autosampler platform proposed in this study integrate the MFX device. Several sample preparation steps immediately before sample injection have been automated, allowing just-in-time sample preparation. Following an example to show the advantages of the use of SPME FFA Multi Off-Line Sampler respect to On-line SPME, we assume an extraction time of 40 minutes -*cis*- and *trans*-CHDA-methyl esters equilibrium in a SPME three phase system (Fig.-5) and analysis time of 20 minutes. The results are excellent, with reduction of the total analysis time of 725 minutes for 30 samples processed (Fig.-6).

In light of what indicated above the authors present the final results. The HHPA efficiency of conversion to its relative carboxylic acids was in accordance with the work-up recovery described by Jonsson et al [25]. In our study, the recovery of the anhydride in alkaline solution was estimated of 81% (CV 3%, 20 ng of HHPA in 100 mg of UPR). As indicated in Table-2 the resulting calibration curves for *cis*- and *trans*-CHDA were linear in the investigated range, showing a correlation coefficients >0.99. Accuracy was within 15% of the theoretical concentration, in line with the requirement of US Food and Drug Administration.

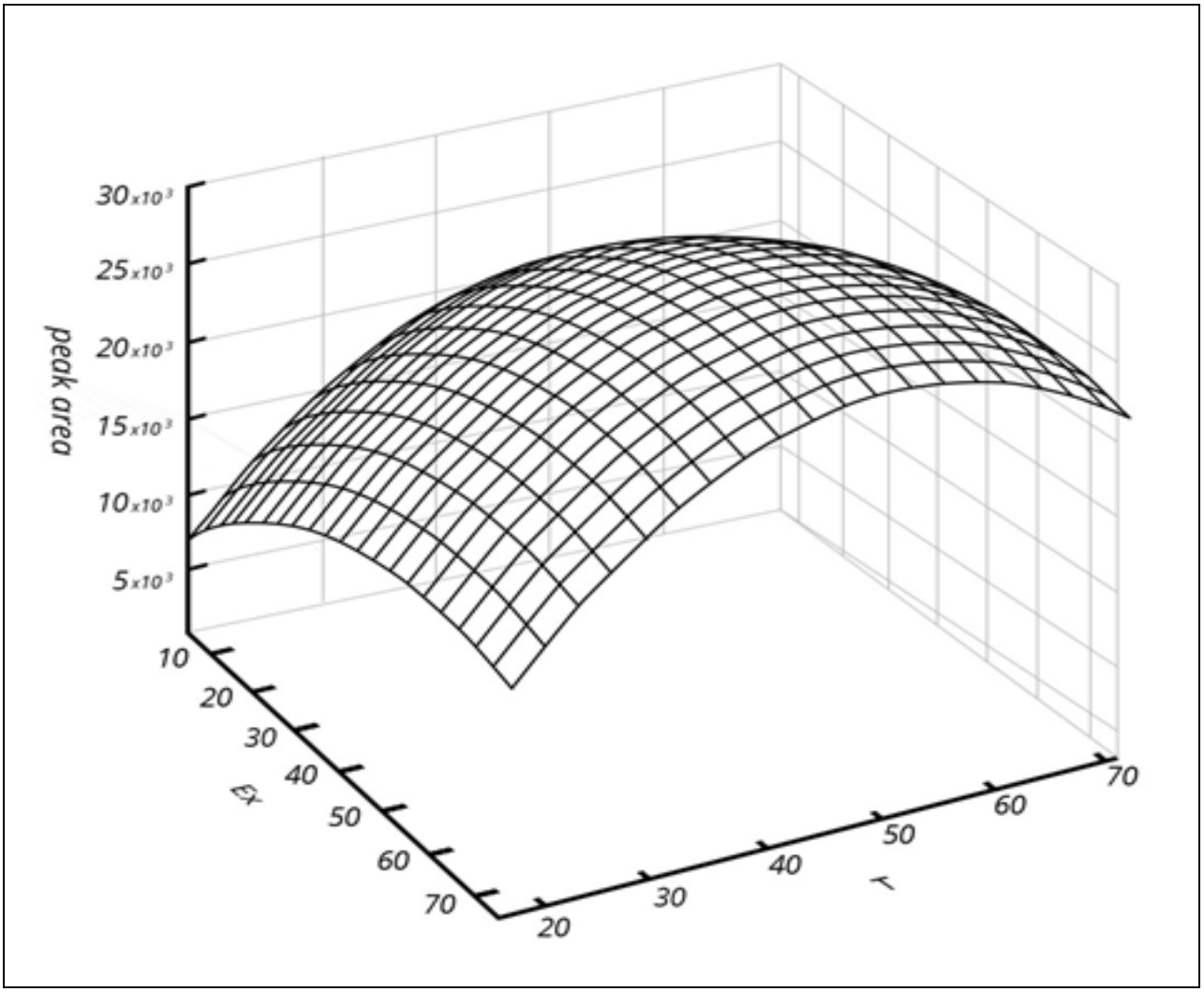
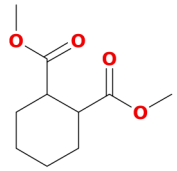



Fig. -5: Response Surface for the dependent variable 'peak area'. Equilibrium time (Eq) was fixed at the center point. Ex (Extraction Time) and T (Extraction Temperature)

Table-1. Physical properties and partition coefficients of CHDA-methyl esters evaluated using SPARC software. The *cis*-(1R, 2S)- (a), *trans*-(1S, 2S)-(b) and *trans*-(1R, 2R)-(c) CHDA-methyl esters isomeric forms, are not distinguished for properties.

| SMILES strings | Structure | CAS No. | $T_{eb}$ (°C) | $D_{water}$ ($cm^2/s$) | $D_{air}$ ($cm^2/s$) | Henry's Constant (atm $m^3$/mol) | $K_{ow}$ (Log) | $P_{vap}$ (log(atm)) |
|---|---|---|---|---|---|---|---|---|
| COC(=O)[C@H]1CCCC[C@H]1C(=O)OC[(a)]<br>COC(=O)[C@H]1CCCC[C@@H]1C(=O)OC[(b)]<br>COC(=O)[C@@H]1CCCC[C@@H]1C(=O)OC[(c)] |  | 3205-35-4 | 270.8 | $6.38*10^{-6}$ | 0.0481 | $5.49*10^{-7}$ | 1.6 | -4.61 |

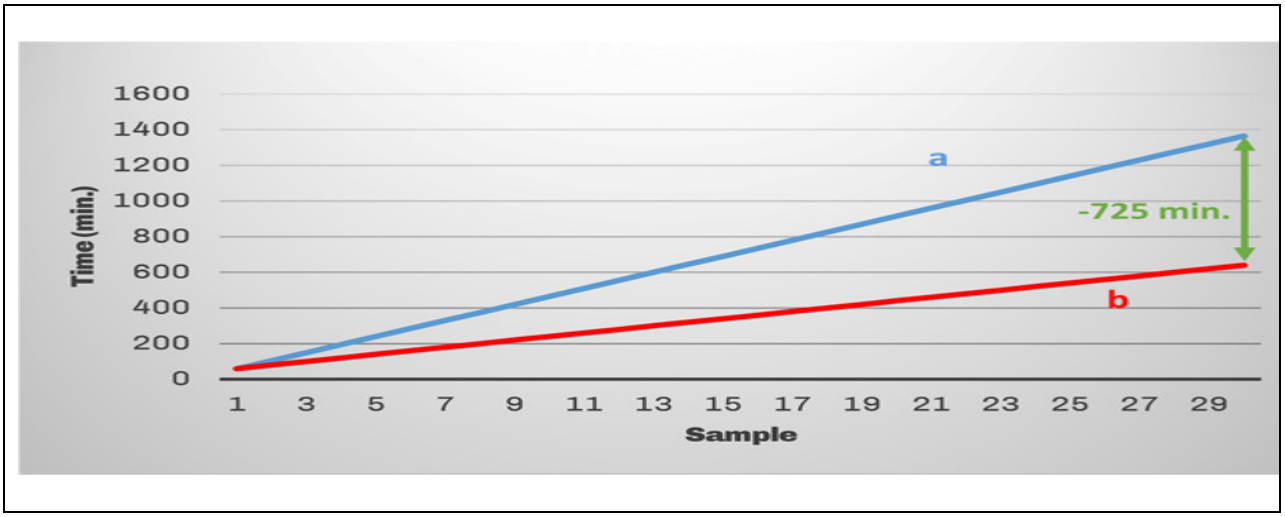



Fig. -6: On-Line (a) vs. Off-Line (b) SPME extraction.

Table-2. Calibration curve. accuracy and precision of *cis*-CHDA and *trans*-CHDA analytical methods.

| Calibration Curve | | |
|---|---|---|
| Nominal concentration of *cis*-/*trans*-CHDA (pg/mg UPR) | *cis*-CHDA measured concentration (pg/mg UPR) Mean (n=5) | *trans*-CHDA measured concentration (pg/mg UPR) Mean (n=5) |
| 25 | 23.7 | 24.9 |
| 50 | 48.8 | 50.7 |
| 100 | 99.7 | 103.7 |
| 200 | 201.9 | 194.4 |
| 400 | 402.7 | 400.7 |
| 800 | 798.8 | 800.5 |
| Response factor plot | | |
| Least-squares linear regression plot parameters (m=slope b=intercept) | m = 0.0636<br>b = 0.1312 | m = 0.0845<br>b = 0.1106 |
| Coefficient of correlation | 0.99 | 0.99 |
| Standard Error | 0.105 | 0.262 |

| Method Detection Limits | | |
|---|---|---|
| LOD (pg/mg) | 2.9 | 8.0 |
| LOQ (pg/mg) | 14.5 | 29.7 |
| Accuracy and Precision | | |
| Within session accuracy (%) | 6.7 | 7.0 |
| Within session repeatability (%) | 7.4 | 8.4 |
| Inter session repeatability (%) | 9.3 | 11.3 |

## CONCLUSION

Our data suggest that automated SPME extraction coupled with GC/MS may be a viable alternative for HHPA analyses in UPR. The introduction of dedicated, automated, and robotic systems allowed a friendly use of MS apparatus for high-throughput screening and it reduces the costs of monitoring campaigns. Lastly, we expanded the application of this methodology by carrying out the methylation of carboxylic acid corresponding to relative methyltetrahydrophtalic and methylhexahydrophthalic anhydrides.

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