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Article

Simultaneous Determination by Selective Esterification of Trimellitic, Phthalic, and Maleic Anhydrides in the Presence of Respective Acids

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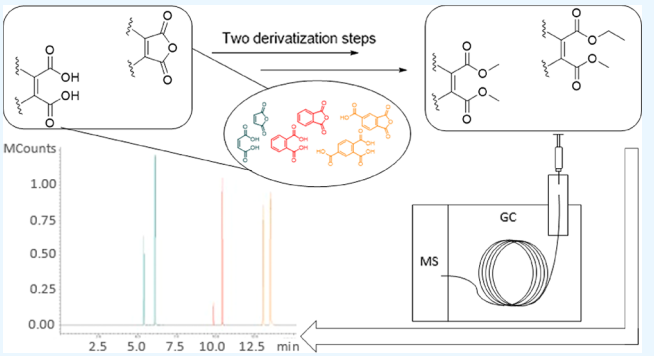
ABSTRACT: Trimellitic, phthalic, and maleic anhydrides are important building blocks to produce polymers and additives, such as plasticizers. In humans, the exposure to these compounds can cause several health issues. In European Union and USA, their presence in substances and mixtures is restricted by CLP Regulation (no. 1272/2008) and HCS/HazCom 2012, respectively, but no information about the corresponding acids is reported. For this reason, a selective method to determine anhydrides in mixtures, in the presence of acids, could be interesting. Nowadays, methods in the literature are either not selective or use explosive and toxic reagents (as diazomethane). In this work, an innovative, greener, and safer method for the simultaneous recognition and quantification of anhydrides and acids, via direct injection gas chromatography−mass spectrometry, is developed. The sample pretreatment consists in selective esterification with absolute ethanol on the anhydride, followed by a treatment with boron trifluoride-methanol for the methylation of remaining carboxylic groups. The optimization of the functionalization, a crucial step of the method, was optimized by experimental design. The limit of detection−limit of quantification (LOD−LOQ) values for trimellitic, phthalic, and maleic anhydrides are 0.31−0.93, 0.47−1.41, and 0.06−0.18 μg/mL, respectively.



INTRODUCTION

According to the definition of the International Union of Pure and Applied Chemistry (IUPAC), a plasticizer is a substance or material that is incorporated into a polymer to increase its flexibility, workability, or distensibility. A plasticizer may also affect other properties, such as the lowering of the glass transition temperature (T_g) and the reduction of the melt viscosity.[1] Poly(vinyl chloride) (PVC) is the polymer more frequently added with plasticizers, obtaining properties that make it widely applied to different sectors, from food packaging to tubing systems, passing through children toys and medical materials.[2]

As far as plasticizer production is concerned, some of the most important building blocks are maleic, phthalic, and trimellitic anhydrides.[3−5] Maleic anhydride (MA), i.e., 2,5-furandione, has a considerable industrial importance due to the presence in the molecule of the olefinic and dicarboxylic anhydride functionalities; therefore, it can be applied for both polyaddition and polycondensation. Besides plasticizers, MA is widely employed in many fields of polymer production, such as copolymers, lubricants, alkyd resins, lacquers, and, as the main application, polyester resins.[3] This results in a worth market of USD 3.4 billion in 2019,[6] and it is expected to cross USD 4.9 billion by 2028 at a Compound Annual Growth Rate (CAGR) of 6.2%.[7] The toxicity of MA for humans is displayed in both acute and chronic exposure modes. Acute exposure to MA can cause irritation of the respiratory tract and eyes, while chronic exposure can cause asthma-like attacks, chronic bronchitis, and upper respiratory tract and eye irritation in workers, as well as allergies in some people; nonetheless, MA is not classified as a carcinogen by EPA.[8] Phthalic anhydride (PA), i.e., isobenzofuran-1,3-dione, was the first anhydride of a dicarboxylic acid to be commercially used. The most important derivatives of PA are plasticizers (mainly for the production of PVC), polyester resins, and dyes.[4] For the period of 2022−2028, the global PA market is expected to register a CAGR of 4.27% due to an increase in the utilization of plasticizers in the Asia-Pacific region and to the growing demand for glass fiber-

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reinforced plastics in various industries.[9] Although PA is not classified as a carcinogen by EPA, short- and long-term exposure effects were observed in humans. Acute exposure to PA does not cause permanent injuries, but it leads to irritation of the skin, eyes, and respiratory tract. Chronic effects observed included rhinitis, rhinoconjunctivitis, bronchitis, conjunctivitis, and irritation of the skin and mucous membranes of the respiratory tract.[10] Trimellitic anhydride (TMA), i.e., 1,3-dioxo-1,3-dihydroisobenzofuran-5-carboxylic acid, is used, to the most part, to make plasticizers for PVC, characterized by lower volatility than phthalate ones. Polyesters and polyimides of TMA have high thermal resistance and are used in the production of wire enamels, baking varnishes, and coatings,[5] even though TMA is much more expensive compared to PA (ca. 90 vs 25 EUR for 1 kg).[11,12] Thanks to its properties, TMA is widely used, and for 2020, the global market size for TMA is estimated at USD 356.5 million and is expected to reach USD 459.2 million by 2027 at a CAGR of 3.7% during the period of 2022−2027.[13] Acute exposure to TMA in humans can cause irritation and burns to the skin and eyes and irritation of the respiratory tract (nose, throat, and lung), and it can cause coughing and shortness of breath. Long-term exposure can cause skin and asthma-like allergies, whereas there is no evidence of carcinogenicity or reproductive hazard.[14]

Since these anhydrides are widely used in industry and they possess health hazards, their presence in manufactures or semifinished products is regulated by institutions. In European Union, the law that controls substances and mixtures is Regulation (EC) No. 1272/2008, also known as CLP Regulation (Classification, Labelling and Packaging):[15] it regulates the content limit in mixtures for TMA and PA as 1000 ppm (0.1%) and for MA as 10 ppm (0.001%), while the corresponding acids (trimellitic (TMAc), phthalic (PAc), and maleic (MAc) acids) are not regulated. In the United States, Occupational Safety and Health Administration (OSHA), in the document HCS/HazCom 2012, establishes the lowest limit for respiratory and skin sensitizer compounds, such as TMA, PA, and MA, at 0.1% (1000 ppm).[16] Nowadays, the validated method to furnish information about the presence of anhydrides is the total acidity:[17] the analysis is performed by titration of 1 g of the plasticizer with a KOH solution, using a colorimetric indicator to observe the variation of the color of the solution, confirming its occurred alkalinization. The result of this analysis, expressed in mg KOH/g product, furnishes information about free carboxylic groups of hydrolyzed anhydrides, free acids, and not totally functionalized esters, without differentiation between the species. For this reason, in the presence of more complex mixtures, in which there is the possibility to have anhydride and acid simultaneously, as well as polymeric compounds made by two different analytes (e.g., maleic-phthalic polyester resins), the possibility to obtain differentiation between these species could be significant in terms of labeling the hazard risk of the final mixture. In this scenario, chromatographic techniques like gas chromatography (GC) and liquid chromatography (LC), often coupled with mass spectrometry (MS), are suitable tools for the analysis of complex mixtures:[18−21] in particular, several analytical methods are reported in the literature for the recognition via GC of cyclic anhydrides.[22] The main strategy, developed in the 80s to 90s, is the esterification of the carboxylic groups using boron trifluoride-methanol (BF_3·MeOH) in an anhydrous environment before the analysis by GC−MS instruments, and it has been successfully and widely applied to TMA,[23,24] hexahydrophthalic anhydride (HHPA),[25,26] and PAc[27] for air and urine samples in the occupational exposure assessment. Other methods used GC coupled with an electron capture detector (ECD) for the analysis of unmodified MA[28] and PA.[29] More recently, trimethyloxonium tetrafluoroborate (TMO) and triethyloxonium tetrafluoroborate (TEO) were applied in aqueous medium for the derivatization of HHPA[30] and TMA,[31] respectively: the first reaction is the hydrolysis of the anhydride to the corresponding acid, followed by esterification. All the methods described above have the limitation of not allowing the simultaneous quantification of both anhydride and the corresponding acid: the reaction with BF_3·MeOH gives the same product. On the other hand, direct injection of unfunctionalized species gives rise to an overestimation of the anhydride, since the acid loses water at the injection temperature, condensing to the corresponding anhydride.[32−34] To bypass this problem, different analytical techniques were taken on account of performing the determination of anhydrides. Purnell and Warwick in 1980 proposed the use of LC for the determination of TMA:[35] also in this case, authors had to hydrolyze TMA to trimellitic acid (TMAc) to be able to perform the analysis. To our knowledge, the only procedure present in the literature achieving simultaneous determination of TMA and TMAc (as impurity) was published in 1982 by Rushing et al.: they derivatized the free carboxylic groups present in both molecules, without functionalizing the anhydride moiety, using diazomethane.[36] However, diazomethane is a highly hazardous reactant: it may explode in contact with glassware,[37] and it is highly toxic by inhalation and eye and skin contact.[38] Even though, in the field of the Green Analytical Chemistry (GAC), the use of chromatographic methods is encouraged, since they are multianalyte methods, the use of hazardous reactants must be replaced, if possible.[39] In this context, a greener method for the simultaneous recognition and quantification of any anhydride in the presence of the corresponding acid could be attractive. In this work, an innovative, fully automated, direct injection GC−MS method is presented: the sample pretreatment consists in selective esterification with absolute ethanol (EtOH), mediated by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), on the anhydride,[40] followed by a treatment with BF_3·MeOH for the methylation of remaining carboxylic groups.[24] The setup of the derivatization process was optimized by experimental design to minimize the amount of tests required. These steps will be discussed, as well as chromatographic aspects and analytical performances.

RESULTS AND DISCUSSION

To simultaneously detect the anhydride and the corresponding acid as esters, a two-step derivatizing method followed by GC−MS analysis was designed.

The first step could be performed by selective esterification in mild conditions: theoretically, anhydrides are more reactive than the corresponding acid in esterification processes; hence, discrimination is possible at this level.[41] In fact, the order of reactivity of carboxylic acids and their derivatives is reported in Figure 1:

The second derivatization process should be done using a different alcohol (MeOH instead of EtOH), in the presence of a catalyst/mediator, to achieve the esterification of all free carboxylic groups not involved in the first derivatization step.

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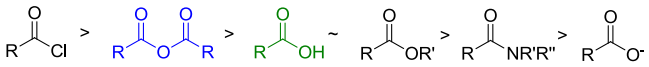
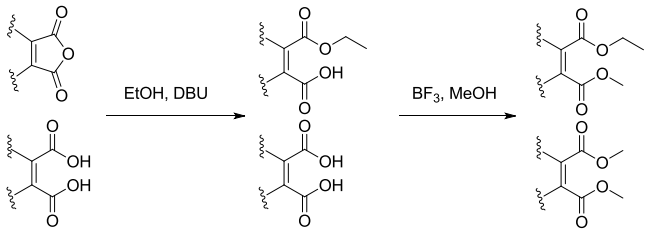


Figure 1. Relative reactivity of carboxylic acids and their derivatives.

The preliminary functionalization test was carried out on TMA using reaction conditions found in the literature: the first step was performed by stirring for 10 min a TMA EtOH solution (1 mg/mL) using an excess of DBU,[40] and then the second step was carried out by heating for 1 h at 100 °C the reaction crude dissolved in a BF_3·MeOH solution.[24] It was found that these conditions were not totally suitable for this analytical method, since trimethyl trimellitate (TMT) was detected in high percentage compared to the analytical target, ethyl dimethyl trimellitate (EDMT). The TMT presence was probably due to a transesterification process in the second step: for this reason, optimization of the conditions was studied. In addition, a mono-step method was also taken into account: the first functionalization is enough to discriminate anhydride and acid, since the reaction is selective on the anhydride, achieving the formation of ethyl trimellitate from TMA and unreacted TMAc. However, tests performed did not show any result in the analytical settings presented in Experimental Section. For this reason, the addition of the second step was necessary.

The two-step procedure was then applied to PA and MA. Working in these conditions, MA/PA and TMA are detected as ethyl methyl ester and ethyl dimethyl ester, respectively, while MAc/PAc and TMAc are detected as di- and trimethyl esters, respectively. These considerations are shown in Scheme 1.

Scheme 1. Multistep Method for the Esterification of Anhydrides and Acids



Derivatization Optimization. Since the preliminary test did not show a proper outcome for TMA in terms of selectivity, the two steps of the reaction were optimized separately: the first one with experimental data only and the second with the aid of an experimental design method.

The first step of the functionalization process was tested by stirring the mixture of TMA, DCM, EtOH, and DBU at room temperature for different times, 10, 20, and 30 min, followed by the second step (heating for 1 h at 100 °C in a BF_3·MeOH solution). The use of DCM as a co-solvent is not mandatory: the reaction was also studied without DCM (replacing it with the same amount of EtOH) with no significant differences, but its presence is useful to treat matrices not totally soluble in EtOH. From an experimental viewpoint, while 10 min of stirring did not guarantee a total conversion of the anhydride in the monoethyl ester, the increase in reaction time to 30 min (or more) showed a small di- and tri-ethyl esterification of the anhydride, lowering the selectivity of the method. The selectivity of the method decreases the improvement of the reaction time, since the reactivity of the free carboxylic group of the monoethyl ester is comparable with that of the corresponding acid: for this reason, theoretically, the formation of diethyl ester from monoethyl ester has the same probability as the formation of monoethyl ester from acid. Hence, reaction times of 30 min (or more) lead to an overestimation of the anhydride amount. Conversely, using 20 min for the reaction step, the performances in terms of sensitivity were improved, compared to 10 min, without loss of selectivity due to further esterification. In the light of these tests, the time chosen for this step was 20 min. In addition, to use the milder condition possible, temperature was not increased. Higher temperatures could improve the esterification process, leading to the esterification of other free carboxylic groups and consequent loss of selectivity between anhydride and acid. Since the reaction was carried out in a high excess of EtOH (solvent and reagent), the management of the reaction time was crucial: in fact, the esterification of more than one carboxylic group was observed for reaction times of 30 min and more. Since the temperature was not managed, only the reaction time is a variable: for this reason, experimental design tests cannot be applied to the first step. With this result in hand, same tests were performed on PA and MA, confirming 20 min as the most suitable reaction time for all the investigated analytes. Moreover, it is crucial to use dry solvents, since water reacts with anhydrides, giving the corresponding acid, leading to an underestimation of the target compound. At the end of the first step, liquid−liquid extraction was necessary to eliminate the excess of DBU: the reaction mixture containing DCM and EtOH was dried under vacuum, and then the resulting semisolid was dissolved in DCM and added with 1 M HCl. The acidification of the solution has two goals: protonating DBU to move it in aqueous medium and protonating the monoethyl-dicarboxylic acid derived from TMA to promote its transition in organic medium. While a higher recovery of the derivative of TMA is useful to improve the sensitivity of the method, the elimination of DBU is mandatory to avoid acid−base reaction between the organic base and BF_3, which would precipitate the Lewis acid: in fact, the excess of this reagent is eliminated in the workup procedure after the reaction, adding pyridine.

Since, in the preliminary test, the transesterification with substitution of −OEt with the −OMe group was observed, probably due to the reaction with BF_3·MeOH, a face-centered central composite design was applied to minimize the number of experiments to reach the optimal conditions in which the transesterification is decreased. This design was adopted to exploit all the possible interactions among the two variables, time and temperature, performing only nine experiments concerning the second derivatization step. The two considered factors and their respective levels are reported in Table 4: the temperatures of the second step at 60, 80, and 100 °C and the reaction times of 10, 20, and 30 min.

On the basis of the experimental design, crudes derived from the first step of derivatization of TMA, PA, and MA were added with a BF_3·MeOH solution and heated at 60, 80, and 100 °C: each temperature was investigated for 10, 20, and 30 min for each anhydride. At the end of the reaction, the solvent was removed, and crudes were dissolved in *tert*-butyl methyl ether (TBME), added with pyridine to achieve the precipitation of a white solid, and centrifuged to isolate liquid phases. Solutions (0.5 mL) were dispensed in 2 mL screw cap vials and added with acetonitrile before the injection in GC.

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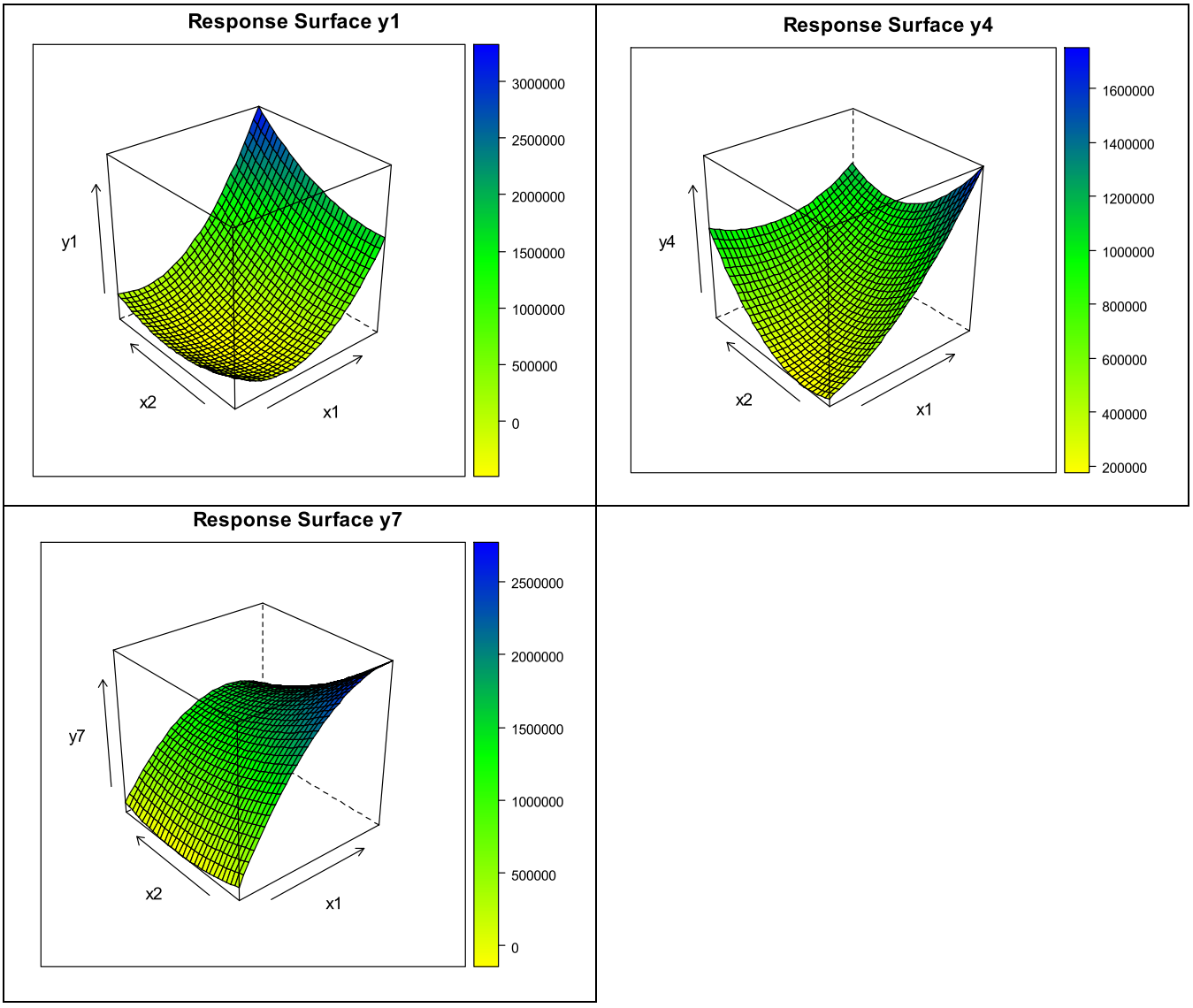

Figure 2. Response surfaces obtained for the methyl-ethyl esters of the three anhydrides. y1 = TMA; y4 = PA; y7 = MA.

Peak areas were integrated by a chromatogram and used for the statistical analyses.

To compute a model, nine responses were studied, namely, the sensitivities (measured by peak areas and indicated by y) of the analytes, correlated with temperature and time, indicated by x1 and x2, respectively. The peak area of ethyl dimethyl trimellitate (EDMT) (y1) and TMT (y2) and their peak area ratio (y3 = y1/y2), that of ethyl methyl phthalate (EMP) (y4) and dimethyl phthalate (DMP) (y5) and their peak area ratio (y6 = y4/y5), and that of ethyl methyl maleate (EMM) (y7) and dimethyl maleate (DMM) (y8) and their peak area ratio (y9 = y7/y8) were studied.

The results obtained (reported in Figure 2) showed that the models describing the sensitivity for the ethyl methyl esters of TMA, PA, and MA were affected mainly by the temperature at which the derivatization was performed, while the time has a minor impact, even though it is crucial in the formation of the di/trimethyl esters. It is evident from this study that, to maximize the sensitivity of EDMT, it was necessary to work at the highest temperature (x1 = 100 °C) and time (x2 = 30 min), while for EMP and EMM, the best conditions met were the highest temperature (x1 = 100 °C) and lowest time (x2 = 10 min).

For TMA, the analysis of the models of the peak area ratio (Figure 3) of EDMT formation and TMT formation suggested that, to maximize the formation of EDMT and minimize that of TMT, the best conditions were 100 °C and 20 min. Concerning PA, the same considerations were suitable, but decreasing the temperature to 80 °C reduces the formation of DMP. Last, for MA, it is necessary to decrease the temperature to 60 °C to avoid the transesterification, even if this could penalize the sensitivity toward EMM.

By the analysis of chromatograms and contour plots (Figure S15), working for 20 min was necessary to reduce transesterification in all cases. Conversely, the best temperature changes for every anhydride, 60 °C for MA, 80 °C for PA, and 100 °C for TMA, evidencing a trend of reactivity in these conditions. In the case of the necessity of multianalyte analyses, it will be necessary to work at the temperature prescribed for the less reactive anhydride to avoid the loss of sensitivity; conversely, a loss of selectivity is observed for the more reactive compound. Tests working in the setup

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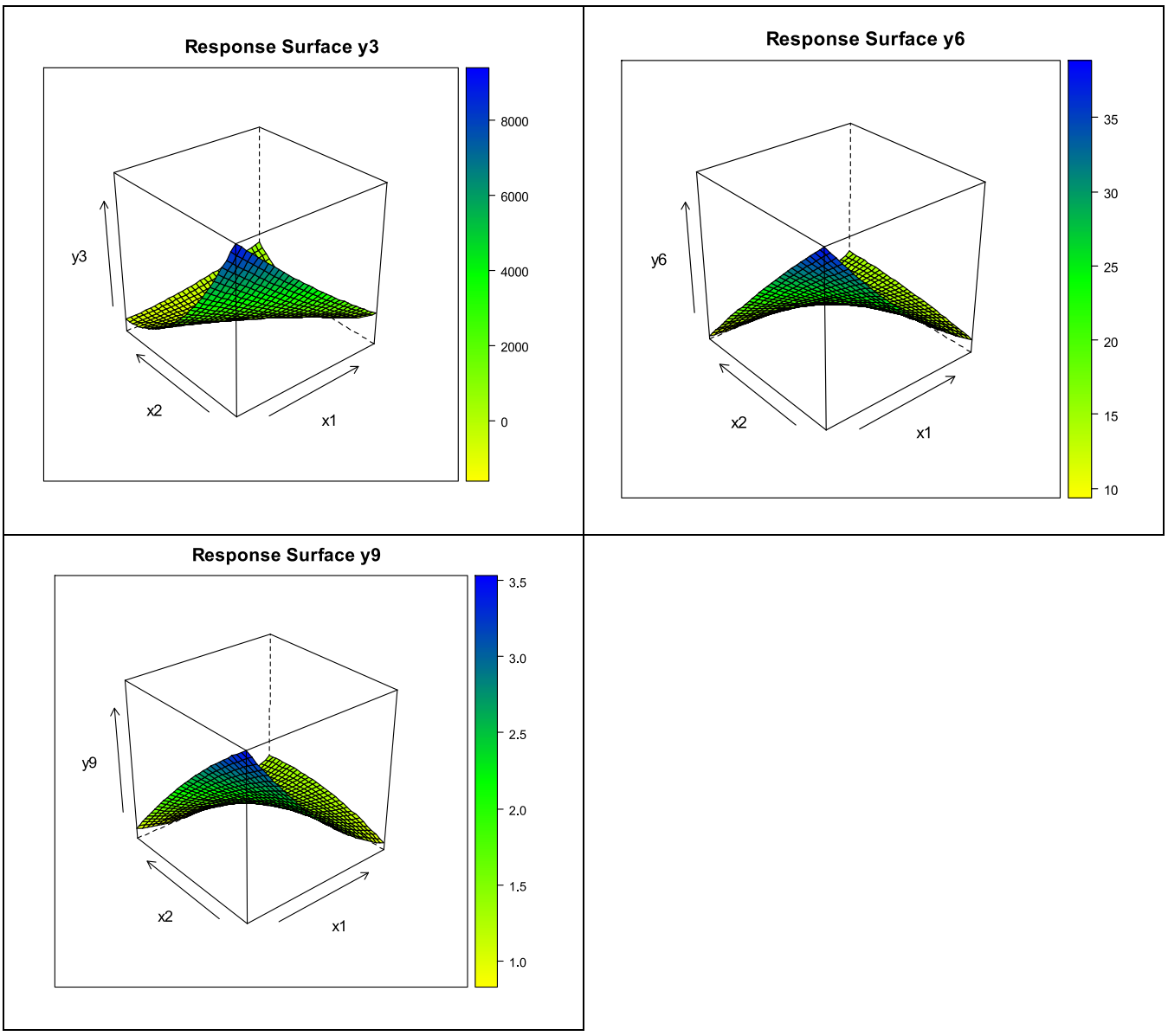

Figure 3. Response surfaces obtained for the ratio between ethyl methyl esters and di/trimethyl esters of the three anhydrides. y3 = TMA; y6 = PA; y9 = MA.

conditions for anhydrides were also performed for the corresponding acids that, as expected, underwent full methylation of their carboxylic groups, obtaining TMT, DMP, and DMM from TMAc, PAc, and MAn, respectively. The selectivity on anhydrides of the first step is a crucial aspect of the method success: in the setup condition, acids are inert. The results for anhydride treatment are shown in Table 1.

Automation of Derivatization and Analysis. High throughput, traceability, minimization of human errors, and safety for operators are the main goals of modern analytical chemistry, and these can be reached by using fully automated procedures. In this work, the fully automated sample preparation and GC injection were performed by a customized *xyz* Autosampler MPSroboticPro Smart Series (Gerstel GmbH & Co.KG, Germany), installed online to the GC instrument. This system allowed one to minimize death times between two samples, resulting in a chromatographic separation with high productivity, permitting a reduction of costs of the analytical assay (the operator has to less assist the process of the analytical platform, and the same number of samples can be processed in less time).[42]

Schematization of the procedure, as well as a picture of the autosampler-GC system, is reported in Figure 4.

Performance Result Assessment. Calibration curve building was designed in the optical of miniaturization: 100 mg of sample can be easily dissolved in 1 mL of the mixture of used solvents. In our analytical conditions, the limit of TMA and PA in the mixture of compounds is 0.1% corresponding to

Table 1. Functionalization Time/Temperature

anhydride	step 1, time (min)	step 2, temperature (°C)	step 2, time (min)
TMA	20	100	20
MA	20	60	20
PA	20	80	20

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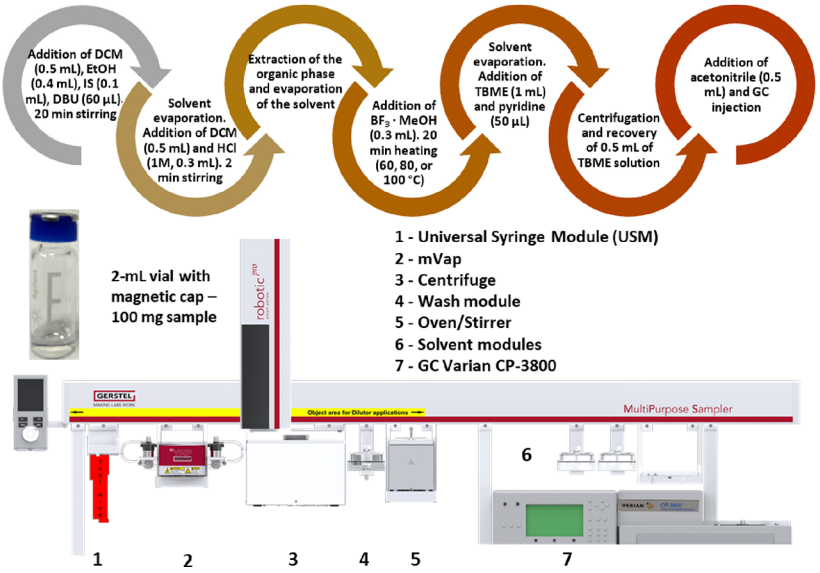

Figure 4. Flow chart and image of the autosampler for the automation of the preparation and analysis procedures (picture courtesy of Andrea Carretta, SRA Instruments SPA, Cernusco sul Naviglio (MI), Italy, free domain).

Table 2. Performance Evaluation of the Method

			RSD (%)		Acc (%)		
anhydride	LOD (µg/mL)	LOQ (µg/mL)	LCP	HCP	LCP	HCP	R^2
TMAn	0.31	0.93	4.7	5.6	97.6	104.8	0.9972
PAn	0.47	1.41	6.2	4.9	102.7	105.5	0.9964
MAn	0.06	0.18	7.8	8.9	94.3	97.4	0.9927

100 µg/mL, while it is 0.001% for MA corresponding to 1 µg/mL.

The limit of detection (LOD) and limit of quantification (LOQ), relative standard deviation (RSD %) (calculated as the standard deviation of the control point divided by its average value and multiplied for 100), accuracy (Acc %) (calculated by the average value divided by the nominal value and multiplied for 100), and R^2 of the regression curve are reported in Table 2. RSD (%) and Acc (%) were evaluated using a low curve point (LCP) and high curve point (HCP), corresponding to 25 (TMA, PA)/0.25 (MA) µg/mL and 175 (TMA, PA)/1.75 (MA) µg/mL, respectively.

CONCLUSIONS

For the first time, an innovative two-step gas chromatographic method for the selective and quantitative analysis of trimellitic, phthalic, and maleic anhydrides in the presence of the corresponding acids has been successfully developed. This method exploits the different reactivity between anhydrides and acids, obtaining a chemical differentiation not affected by high temperature (able to convert acid into anhydride) nor water presence (hydrolysis of anhydride with formation of acid). The evaluation of the method performances confirms its suitability for the analysis of these analytes, since LOD and LOQ are lower than the regulated limit in mixtures, and precision and accuracy are acceptable. Last, the method was developed in a fully automated procedure, permitting savings in both time and cost, improving the safety of operators, and reducing human errors during the procedure.

EXPERIMENTAL SECTION

Chemicals and Reagents. Maleic anhydride (MA) (CAS 108-31-6), maleic acid (MAc) (CAS 110-16-7), phthalic anhydride (PA) (CAS 85-44-9), phthalic acid (PAc) (CAS 88-99-3), trimellitic anhydride (TMA) (CAS 552-30-7), trimellitic acid (TMAc) (CAS 528-44-9), phthalic anhydride-d_4 (CAS 75935-32-9), ethanol (EtOH) (CAS 64-17-5), dichloromethane (DCM) (CAS 75-09-2), *tert*-butyl methyl ether (TBME) (CAS 1634-04-4), pyridine (CAS 203-809-9), acetonitrile (CAS 75-05-8), boron trifluoride-methanol solution, 14% in methanol (BF_3·MeOH) (CAS 373-57-9), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (CAS 6674-22-2), and 1 M hydrochloric acid solution (in water) (CAS 7647-01-0) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). The gas helium (99.999%) was obtained from Air Liquid (Paris, France). Clear 2 mL screw vials (Part No. 5182-0714, Agilent Technologies, Santa Clara, CA, USA) were fitted with screw thread caps for magnetic transport (Thermo Fisher Scientific, Waltham, MA, USA, cat. no. 9-MSC(BG)-ST101).

Instruments. A Varian CP3800 GC with two 1078−1079 injector ports was coupled with a mass spectrometer Varian Saturn 2200 Ion-Trap. The injection was carried out in split mode (1:10; split flow, 20 mL/min) and with the injector temperature set at 230 °C. The chromatographic column was a DB 35-MS-UI GC Column (30 m × 0.25 mm, 0.25 µm). The initial temperature column was set to 60 °C (0.5 min) and then increased by 15 °C/min to 250 °C, which was held for 8 min. Helium, as the carrier gas, was set at 1.0 mL/min. Full automation of the procedure is described in Online Robotic System (see below). The m/z acquisition window was 50−300, and the MS filament was on from 3 to 18 min.

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Online Robotic System. Automation of the analytical procedure was achieved using an Autosampler MPSroboticPro Smart Series (Gerstel GmbH & Co.KG, Germany). The apparatus was equipped with a GERSTEL-mVap-Option 2, centrifuge for 4 × 2 mL vials (max. acceleration, 2000*g*), agitator (with an adaptor for 2 mL vials), Universal Syringe Module (USM) for syringes from 1 μL up to 1000 μL, gripper, 10 μL, 100 μL, and 1 mL GERSTEL Smart TriStar Syringe for MPS robotic USM or MPS liquid, three solvent modules for 3 × 100 mL solvent bottles, and wash module.

Sample Preparation. Samples (100 mg) were dispensed in a 2 mL vial and posed on the autosampler. Then, DCM (0.5 mL), EtOH (0.4 mL), IS solution (0.1 mg/mL phthalic anhydride-d_4 in EtOH, 0.1 mL), and DBU (60 μL) were added, and the solution was vigorously stirred for 20 min before evaporating the solvent under vacuum. The remaining phase was dissolved in DCM (0.5 mL) and added with 1 M HCl (0.3 mL) to eliminate DBU by liquid−liquid extraction; DCM solution was recovered in a second 2 mL vial and dried under vacuum. The residual phase was subsequently dissolved in BF_3·MeOH (0.2 mL) and heated for 20 min in an oven at 60, 80, and 100 °C to functionalize MA/MAc, PA/PAc, and TMA/TMAc, respectively, and then the sample was left to cool to room temperature and dried under vacuum. Finally, it was dissolved in *tert*-butyl methyl ether (TBME), added with pyridine (50 μL), and centrifuged for 5 min at 3000 rpm. The liquid phase was partially recovered (0.5 mL) in a third 2 mL vial and added with acetonitrile (MeCN) (0.5 mL) for the analysis via GC−MS (Table 3).

Table 3. GC−MS Parameters of Analytes

compound	retention time (min)	qualifier ion (*m*/*z*)	quantifier ion (*m*/*z*)
dimethyl maleate	5.39	113	113
ethyl methyl maleate	6.11	127	113
dimethyl phthalate	9.88	163	163
ethyl methyl phthalate	10.43	177	163
trimethyl trimellitate	12.88	221	221
ethyl dimethyl trimellitate	13.29	235	221

Calibration Curve Construction. Standard solutions (1 mg/mL) were prepared by dissolving the analyte (2 mg) in EtOH (2 mL). For MA, a 0.01 mg/mL solution was prepared by diluting 1 mg/mL and used for the preparation of the calibration curve. Calibration solutions were prepared in the same way for all the investigated anhydrides: to 0.5 mL of dichloromethane (DCM), a proper volume of standard solution was added before mixing with EtOH up to 1 mL. Then, the procedure followed the one described for samples. MA calibration points were 0.01, 0.05, 0.1, 0.5, 1.0, 1.5, and 2.0 μg/mL, while PA and TMA calibration points were 1, 5, 10, 50, 100, 150, and 200 μg/mL.

Experimental Design. The data were collected using Microsoft Excel and processed using Chemometric Agile Tool (CAT), an open-source and R-based software.[43]

A face-centered central composite design (FCCD) was applied: two factors (temperature and time of the reaction) studied at three levels for each one. Table 4 briefly reports the experiments performed.

Method Performance Evaluation. To evaluate the precision and accuracy of the method, two control solutions (1 mL) for each anhydride (corresponding to 0.25 and 1.75 μg/mL for MA and 25 and 175 μg/mL for PA and TMA) were prepared and analyzed, following the procedure described above. To assess interday performances of the method, three different sets of calibration and standard solutions were arranged for each anhydride and analyzed on six different days, and average curves were built daily. Six different sets of calibration and standard solutions were prepared and analyzed sequentially to estimate intraday performances. The calibration curves were attained by plotting the peak area of ethyl esters versus the nominal concentration of each calibration solution. To get the best fitting function between the calibration points, least-squares linear regression analysis was employed. The standard deviation (SD) of the response and slope approach was applied to obtain reliable LOD and LOQ values. In fact, the value of LOD was strongly influenced by the stability and reproducibility of the background noise when LOD was checked as a signal-to-noise (S/N) evaluation approach. Therefore, by the standard deviation of *Y*-intercepts (SDY-I) of regression curves, it was possible to calculate the estimated SDs of responses. A relative standard deviation (RSD %) approach was used to evaluate the precision of the quantitative data of the replicate analysis of the control solution. The calculation of the yield between the determined and nominal amounts of the control solution permitted the determination of the accuracy.

Table 4. Experimental Matrix with the Corresponding Experimental Plan

	experimental matrix		experimental plan	
exp#	x1	x2	*T* (°C)	*t* (min)
1	−1	−1	60	10
2	0	−1	80	10
3	1	−1	100	10
4	−1	0	60	20
5	0	0	80	20
6	1	0	100	20
7	−1	1	60	30
8	0	1	80	30
9	1	1	100	30

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c00659.

Chromatograms and mass spectra of the investigated compounds and contour plots of model-computing peak areas (PDF)

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Notes

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