Direct Electrospray Ionization Mass Spectrometry Quantitative Analysis of Sebacic and Terephthalic Acids in Biodegradable Polymers

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Direct Electrospray Ionization Mass Spectrometry Quantitative Analysis of Sebacic and Terephthalic Acids in Biodegradable Polymers

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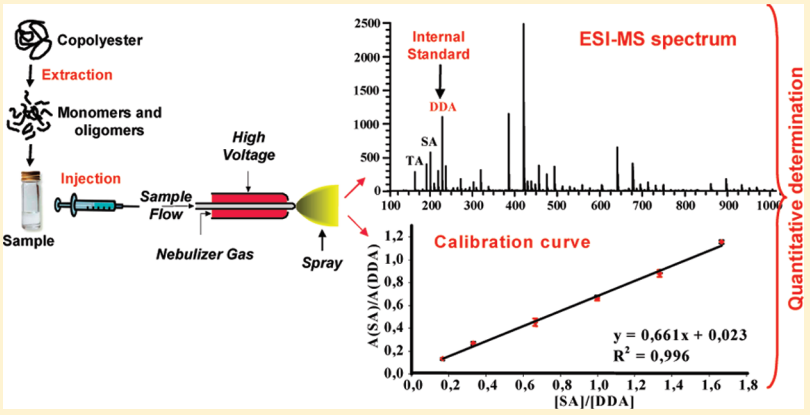
Supporting Information

ABSTRACT:



A direct, rapid, and easy electrospray ionization mass spectrometry (ESI-MS) method to determine concentrations of sebacic acid (SA) and terephthalic acid (TA) residues in biodegradable copolymers was developed. Copolyester samples were synthesized from 1,4-butanediol and sebacic and terephthalic acids by melt polymerization. Extraction of monomers was performed in methanol. Their concentrations were determined by direct infusion ESI-MS, without chromatographic separation, using 1,12-dodecanedioic acid (DDA) as an internal standard. Calibration curves were obtained by plotting the ratio of the areas of the peaks relative to monomers and DDA standard as a function of their concentration ratio. We validated the method by determining the concentration of TA residue using both the ESI-MS protocol and high-performance liquid chromatography (HPLC) analysis with UV detection. The linearity range and the detection limit of this assay were 0.1−5.0 and 0.01 ppm for SA and 0.1−6.0 and 0.03 ppm for TA. This assay represents a useful alternative to conventional methods currently employed for acid quantification, resulting advantageous for its speed and high sensitivity.

The environmental pollution and the solid-waste management related to the significantly growing production of plastics, together with their durability, have stimulated in the last decades increasing interest in biodegradable polymers as green materials. Within this group of environmentally friendly polymers, polyesters play a major role due to their potentially hydrolyzable ester bonds. Aromatic polyesters such as poly(ethylene terephthalate) (PET) and poly(butylene terephthalate) (PBT) display excellent material properties but proved to be resistant to microbial attack. On the other hand, many aliphatic polyesters turned out to be biodegradable[1−3] but do not cover optimal thermal, mechanical, and processing properties, and this reduces in general their industrial applications. Aliphatic−aromatic copolyesters have been developed as biodegradable polymers for many years[4−18] to combine good material properties, end-use, processing facilities, and suitable biodegradability. Research papers on the syntheses,[4−7] characterization,[4,5,8,9] and biodegradation behavior[4,7,10−14] of aliphatic−aromatic copolyesters have been reported. Witt et al. published data of copolyesters of ET (ethylene terephthalate), propylene terephthalate (PT), and BT (butylene terephthalate) with adipic acid (AA) and sebacic acid (SA), where statistically copolyesters with a content of terephthalic acid (TA) of up to about 50 mol % were degraded in a compost simulation test at 60 °C.[18] BASF and DuPont commercialize aromatic copolyesters with Ecoflex[19] and Biomax trade marks, respectively. Biomax has a high terephtalic acid content which modifies some properties such as the melting temperature (200 °C). According to Muller and co-workers,[7,9,12,13,18] copolyesters with about 35−55 mol % of terephthalic acid are in the most favorable range that guarantees biodegradability and suitable mechanical and physical properties. They also observed[13] that biodegradation of polymers containing aromatic dicarboxylic acids depends on the sequential arrangement

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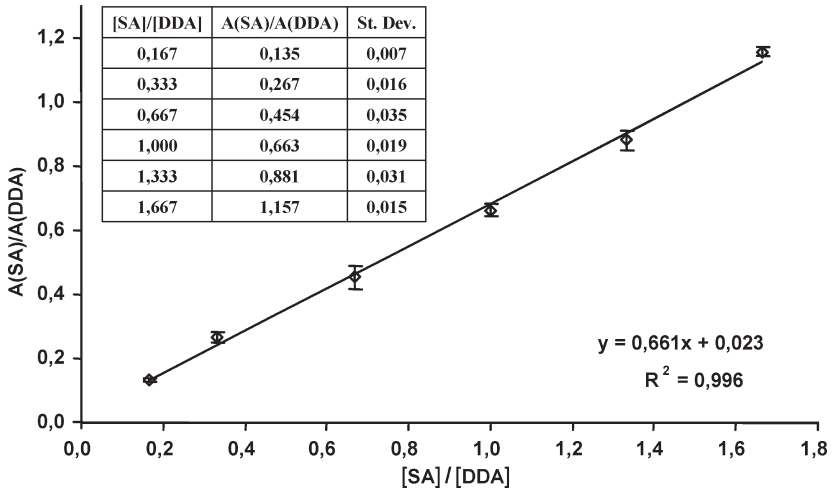

Figure 1. Calibration curve for sebacic acid obtained by direct ESI-MS assay using the internal standard method.

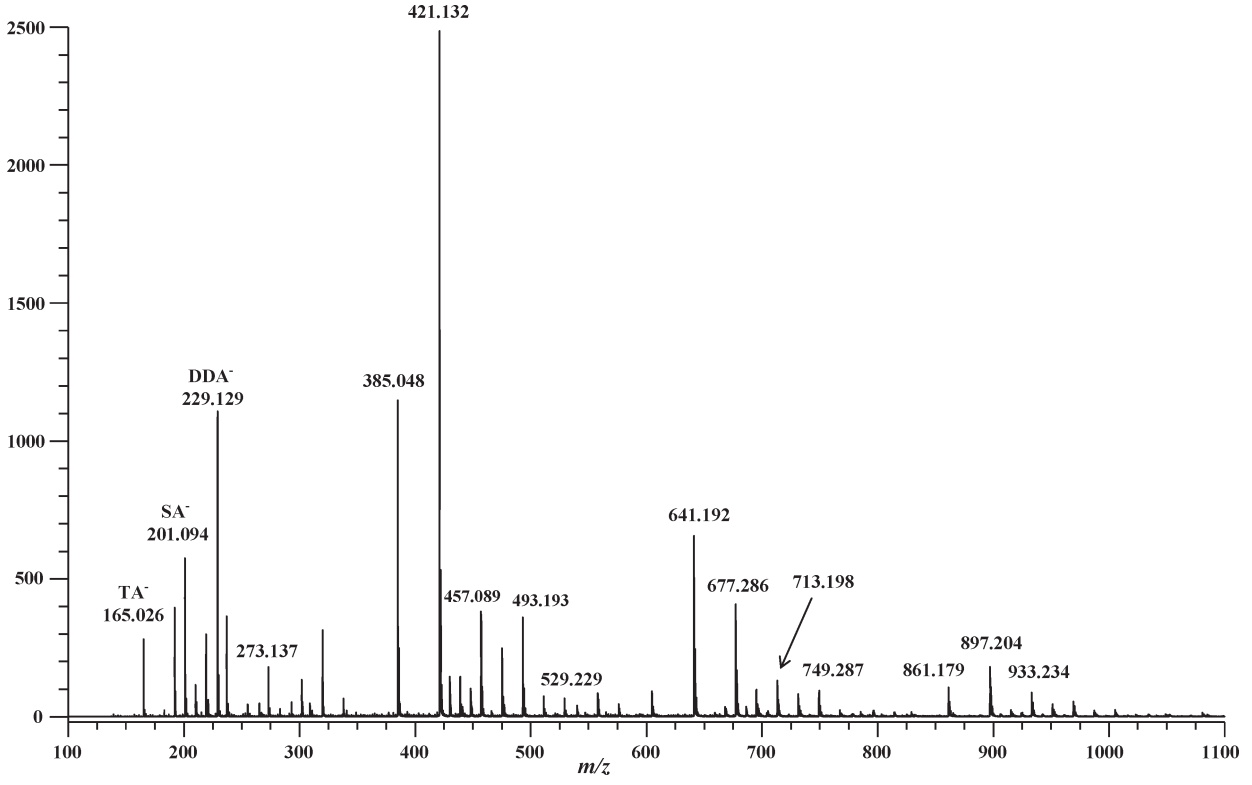

Figure 2. ESI-MS spectrum, acquired in negative ion mode, of monomers and oligomers extracted from sample 1.

of the aromatic units. Complete biodegradation of oligomers with 1 or 2 adjacent aromatic sequences occurred within 4 weeks, whereas oligomers with three adjacent aromatic sequences showed very little degradation over a period of several months. Aliphatic—aromatic copolyesters can be used in agricultural and sanitary fields as well as in packaging applications.

However, residues of monomers used to make food contact plastics can remain not reacted in finished plastics. Residual monomers may then migrate from plastic end products into food. Specific migration limits and maximum permitted quantity of substances in the finished materials are established by environmental directives that can be different according to country legislation. Identification and quantification of monomers, oligomers, or other products, derived from degradation processes, are essential because these compounds might have unsafe effects on human health. Consequently, new, simple, and fast, analytical methods to identify and quantify residue of monomers and oligomers are welcome. Mass spectrometry (MS) has numerous advantages for analyzing several classes of compounds even in complex mixtures, including high sensitivity, selectivity, and speed.[20−24] Electrospray ionization (ESI) is an extensively used ionization method in MS. Its relative simplicity, ease of coupling with liquid chromatography (LC), and usability for a large number of analytes have made it the primary ion source for today's LC-MS instruments.[25−28] The abundances of ions in ESI mass spectra are related to various factors. Molecules that are more basic,

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Table 1. Structural Assignment of ESI Mass Spectrum, Acquired in Negative Ion Mode, Reported in Figure 2

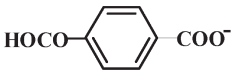
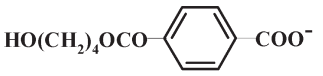
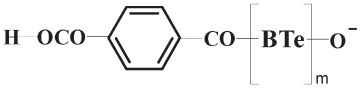
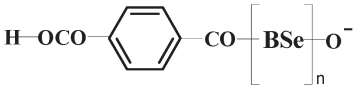
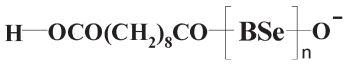
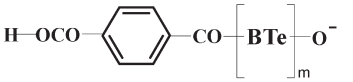
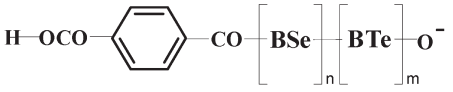
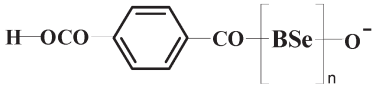
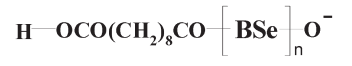
Symbols	Structure	n	m	Measured *m/z*	Calculated *m/z*
TA^-		/	/	165,018	165,026
SA^-	$HOCO(CH_2)_8COO^-$	/	/	201,112	201,094
DDA^-	$HOCO(CH_2)_{10}COO^-$	/	/	229,144	229,129
$(BTe)^-$		/	1	237,076	237,054
$(BSe)^-$		1	/	273,170	273,137
$Te(BTe)^-$		/	1	385,091	385,048
$Te(BSe)^-$		1	/	421,185	421,132
$(BTe)_2^-$		/	2	457,146	457,089
$Se(BSe)^-$		1	/	457,279	
$(BTe)(BSe)^-$		1	1	493,243	493,193
$(BSe)_2^-$		2	/	529,337	529,229
$Te(BTe)_2^-$		/	2	605,164	605,091
$Te(BSe)(BTe)^-$		1	1	641,258	641,192
$(BTe)_3^-$		/	3	677,223	677,286
$Te(BSe)_2^-$		2	/	677,352	
$(BTe)_2(BSe)^-$		1	2	713,316	713,198
$Se(BSe)_2^-$		2	/	713,446	
$(BSe)_2(BTe)^-$		2	1	749,410	749,287

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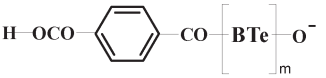
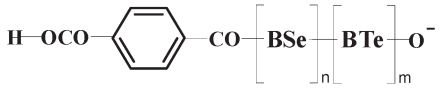
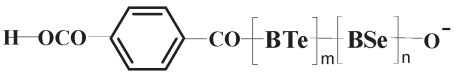
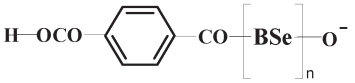
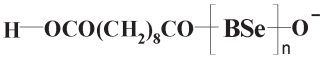
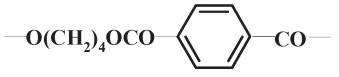
Table 1. Continued

Symbols	Structure	n	m	Measured *m/z*	Calculated *m/z*
$(BSe)_3^-$		3	/	785,503	785,370
$Te(BTe)_3^-$		/	3	825,237	825,146
$Te(BSe)(BTe)_2^-$		1	2	861,331	861,179
$Te(BTe)(BSe)_2^-$		2	1	897,425	897,204
$(BTe)_4^-$		/	4	897,297	
$(BSe)(BTe)_3^-$		1	3	933,388	933,234
$(BSe)_3Te^-$		3	/	933,519	
$(BSe)_2(BTe)_2^-$		2	2	969,483	969,313
$(BSe)_3Se^-$		3	/	969,613	
$(BSe)_3(BTe)^-$		3	1	1005,576	1005,386
$(BTe)_5^-$		/	5	1117,371	1117,425
$Se(BSe)(BTe)_3^-$		1	3	1117,498	
$(BSe)(BTe)_4^-$		1	4	1153,462	1153,511
$Se(BSe)_2(BTe)_2^-$		2	2	1153,592	
$Se(BSe)_3(BTe)^-$		3	1	1189,686	1189,482
$(BTe)_3(BSe)_2^-$		2	3	1189,556	

BSe = —$O(CH_2)_4OCO(CH_2)_8CO$—; BTe = 

or those showing high surface activities or hydrophobicity, are more readily ionized,[29−34] and the ionization efficiency may depend on analyte conformation and concentration, as well as on solution composition or matrix.[35−38] Mass-dependent ion transmission and detection also influence measured ion abundances.[33] Consequently, ESI is usually used for the quantitative analysis of mixtures in tandem with chromatographic techniques, mainly high-performance liquid chromatography (HPLC). However,

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methods for the direct quantification of different classes of compounds by electrospray ionization mass spectrometry (ESI-MS) or ESI MS/MS have been developed.[39−43]

Traditional methods for the quantification of analytes include GC/MS and HPLC, which are labor intensive and time-consuming, and therefore, the capability to obtain rapidly quantitative information about the analyte concentrations directly from mass spectra is desirable. In this paper, we describe a new, rapid, and easy method that enables accurate quantification of not reacted SA and TA monomers from biodegradable copolyesters.

EXPERIMENTAL SECTION

Materials. Terephthalic acid (TA), sebacic acid (SA), 1,12-dodecanedioic acid (DDA), titanium(IV) butoxide, and solvents were purchased from Sigma-Aldrich (MI, Italy), whereas 1,4-butanediol was obtained from Janssen Chimica. Reagents were purified before use, except for 1,12-dodecanedioic acid, which was of the highest grade. Copolyesters, poly(butylene sebacate-*co*-butylene terephthalate) 45/55, P(BSe-*co*-BTe) 45/55, were synthesized by melt polycondensation starting from terephthalic acid, sebacic acid, and 1,4-butanediol, in the presence of titanium-(IV) butoxide.[44] Copolymers were characterized by size exclusion chromatography (SEC) and ^{1}H NMR spectroscopy.

Characterization Methods. *SEC Analyses.* SEC analyses were carried out in $CHCl_3$ with a Waters 515 HPLC pump, equipped with four Ultrastyragel HR columns (in the order HR4, HR3, HR2, and HR1) connected in series, and a Waters R401 differential refractive index detector. Polymer solutions (100 μL, 1 mg/mL) were injected and eluted at a flow rate of 1 mL/min. Polymer Lab Caliber software was used to compute the average molar masses of all the samples by means of the calibration curve obtained using a set of primary polystyrene standards.

NMR Spectroscopy. ^{1}H NMR spectra of polymer samples were recorded at 500 MHz on a Varian Unity *INOVA* spectrometer in $CDCl_3$ at 27 °C. Spectra were acquired with a spectral width of 5000 Hz in 32K data points, an excitation pulse of 60°, and an acquisition time of 6 s, processed using WINNMR (Bruker), and referenced with internal tetramethylsilane (TMS). Copolymer composition, as measured by ^{1}H NMR, was found to be almost equal to the feed ratio used in the synthesis.

Extraction of Monomers. Two copolyester samples with the same composition but different molecular weight (sample 1: M_w = 110 000 g/mol, M_n = 38 000 g/mol; sample 2: M_w = 76 000 g/mol, M_n= 32 000 g/mol) were selected for the extraction of the residual monomers. Extraction of monomers was performed in two steps: each sample (4 g) was dissolved in the minimum amount of $CHCl_3$ (about 20 mL for sample 1 and 16 mL for sample 2) and precipitated into methanol (ratio V_{CHCl3}/V_{CH3OH} = 1/10) (step 1). The solid polymeric material was filtered off and washed with CH_3OH. The methanol solution, containing residual catalyst, monomers, and oligomers, and the washings were combined and brought to dryness with a Buchi RII 2411 V0 rotavapor, using a Buchi V/700 vacuum pump. Monomers were extracted from the dried powdery material using 15 mL of CH_3OH, under stirring for 24 h at room temperature (step 2). Three independent precipitation/extraction procedures were repeated for each sample to determine the associated error. The extraction solution was analyzed, after centrifugation, by HPLC and ESI/TOF-MS. Step 2 was repeated twice in order to verify the efficiency and completeness of the extraction.

Table 2. Sebacic Acid Concentrations Determined by Direct ESI-MS Assay in Samples 1 and 2

	SA (mg/L) by ESI	
replicate	sample 1	sample 2
1	4.78	2.10
2	4.28	2.13
3	4.86	2.05

Three cycles of dissolution/precipitation (step 1) and extraction (step 2) were performed on the same aliquot of sample, finding only negligible amounts of monomers in the final extraction. To validate the extraction method, known amounts of monomers were added to a polymer sample obtained by two successive cycles of dissolution and precipitation. After further precipitation (step 1) in methanol, monomers were extracted (step 2) as mentioned above and quantified by HPLC and/or ESI-MS.

HPLC Analysis. Monomers and oligomers were separated by a HPLC system, equipped with two pumps (Knauer K1001, Berlin, Germany) and a dynamic mixer (Knauer), and connected to an UV detector (Varian 2050), set at 254 nm, or an Evaporative light scattering detector (PL-ELS 1000, Labservice). The Clarity software (Data Apex, Repubblica Ceca) was used for data collection and processing. Injection volume was 20 μL. Separation was carried out at 30 °C using a 5 μm Vydac DENALI (LabService, Italy) C_{18} column (100 × 3.2 mm). Elution was performed at a flow rate of 0.5 mL/min using a gradient of 0.05% formic acid and CH_3OH (HPLC-grade solvents) within 16 min. The first elution step consisted of a linear gradient from 20% to 60% of CH_3OH in 5 min, followed by an isocratic elution for 8 min and a linear gradient from 60% to 100% of CH_3OH in 3 min. The concentration of TA was calculated via integration of the peak areas by the external standard method.

ESI/TOF Mass Spectrometry Analysis. ESI-TOF spectra were acquired by a Mariner Biospectrometry Workstation (*PerSeptive Biosystems*), equipped with an API (atmospheric pressure ionization) source. Mass spectra were recorded working in negative ion mode in the mass range of m/z 100−1200, with 3 s acquisition per spectrum. The spray tip potential was 3 KV, and the nozzle potential was 130 V. The quadrupole interface and the nozzle temperature were set at 140 °C. Nitrogen was used as a nebulizer gas with a flow rate of 0.4 L/min. The mass scale was calibrated with a mixture of angiotensin, bradykinin, and neurotensin. Resolution was about 3500 fwhm. The Data Explorer software was used for the evaluation of the data (computation of ion peak area). For analysis, 20 μL of sample was injected (direct injection) into the mass spectrometer, using methanol/acetonitrile (90:10, v/v) as solvent at a flow rate of 10 μL/min (Harvard Apparatus syringe pump).

Concentrations of SA and TA were determined using the internal standard method. DDA was used as an internal standard. A calibration curve was obtained by plotting the ratio of the areas of the peaks relative to the acid and the DDA standard as a function of their concentration ratio.

RESULTS AND DISCUSSION

Monomers and oligomers extracted from samples 1 and 2 were separated by HPLC and revealed by UV at 254 nm. SA concentration resulted in lower than the concentration limit detectable by UV.

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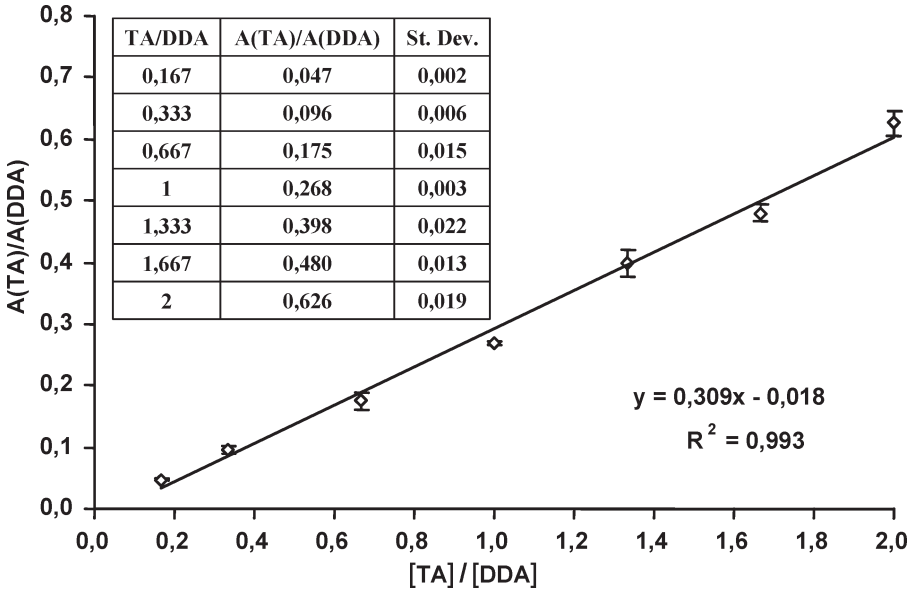

Figure 3. Calibration curve for terephthalic acid obtained by direct ESI-MS assay using the internal standard method.

An ELS (evaporative light scattering) detector was also tested unsuccessfully as an alternative.

Because of its high sensitivity and selectivity, direct ESI-MS assay using the internal standard (IS) method was investigated to replace HPLC analysis for the quantification of SA. Mixtures of SA and DDA in CH_3OH/CH_3CN 90/10 were injected in the ESI source. Increasing concentration of SA, from 0.5 to 5 ppm, and a fixed concentration of DDA (3 ppm) were used. For each mixture, the ratio of the areas of the peaks at $m/z = 201$ [A(SA)] and $m/z = 229$ [A(DDA)], corresponding to deprotonated sebacic and 1,12-dodecanedioic acids, was calculated. The ratio of the areas of the two peaks, A(SA)/A(DDA), was plotted as a function of their concentration ratio, [SA]/[DDA], and the six-point calibration curve, reported in Figure 1, was obtained. Four different solutions with the same concentration ratio, [SA]/[DDA], were prepared and analyzed in order to estimate the error associated with the sample preparation. Replicate injections of the same analyte solution have been carried out as well, and the reproducibility of the MS analysis was higher than that of the extraction procedure (standard deviation ≤ 0.01).

The concentration range for the calibration curve was chosen on the basis of the unknown analyte. Below 0.1 ppm and above 5 ppm, the calibration curve lost its linearity, and the detection limit was 0.01 ppm.

Each point of the curve is the mean value of the four independent ESI measurements. A low standard deviation and a good correlation coefficient were achieved. Obviously, the spectrometer settings, such as spray tip potential, nozzle potential and temperature, etc., influence the calibration with the internal standard. In this case, a tuning of the mass spectrometer was carried out to maximize the sensitivity in the low mass range. A major influence of the spray tip potential on the response of the analytes was observed.

In Figure 2, a representative ESI mass spectrum, acquired in negative ion mode, of the monomers and oligomers extracted from sample 1 is shown. DDA was added in the mixture as an IS. Peaks extend up to m/z 1100 and are due to singly or doubly charged ions (Table 1). Ions at $m/z = 165.069$, 201.107, and 229.087 correspond to TA, SA, and DDA, respectively.

Table 3. Terephthalic Acid Concentrations Determined by HPLC and by Direct ESI-MS Assay in Samples 1 and 2

	TA (mg/L)			
	sample 1		sample 2	
replicate	HPLC	ESI	HPLC	ESI
1	4.88	4.63	3.80	3.65
2	4.65	4.84	3.73	3.80
3	4.83	5.08	3.79	3.72

The concentration of SA was calculated using the equation of the calibration curve in Figure 1. For both samples, three independent replicate measurements on the mixture of oligomers and monomers obtained from three precipitation/extraction procedures were carried out, and the results are reported in Table 2.

The standard deviations of samples 1 and 2 were 0.31 and 0.04, respectively. The difference among the three independent replicate measurements of each sample is related not only to the analytical method but also to the different molecular weight and viscosity of the two samples that could affect the efficiency and reproducibility of the precipitation/extraction procedure.

In order to validate the direct ESI quantification method, we determined the concentration of terephthalic acid residue using both the ESI-MS protocol and HPLC analysis with UV detection. Concentration of TA was determined by HPLC using the external standard method (Supporting Information, Figure S1). The calibration curve obtained by ESI, plotting the ratio of the areas of the peaks relative to TA and the DDA standard as a function of their concentration ratio, is shown in Figure 3. Again, the concentration range has been chosen on the basis of the unknown analyte. However, below 0.1 ppm and above 6 ppm of TA, the calibration curve lost its linearity, and the detection limit was 0.03 ppm.

In the case of TA, the R^2 coefficient (0.993) was not as good as the value obtained for the calibration curve of SA ($R^2 = 0.996$). Reasonably, this minor difference should be related to the nature of the internal standard selected. DDA is more similar to SA than

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to TA. As a general rule, in all calibration methods with internal standard, the more the IS is chemically close to the compound that is to be quantified, the more reliable are the results.

In Table 3, concentrations of TA, calculated from HPLC and ESI assays, for both samples 1 and 2 are compared. The values obtained by the two methods are in good agreement and, again, the concentrations calculated for sample 2 present a lower standard deviation (s_{HPLC} = 0.04, s_{ESI} = 0.08) in comparison with the value of sample 1 (s_{HPLC} = 0.12, s_{ESI} = 0.23). This small discrepancy, evidenced using both HPLC and ESI assays, could be related to the higher molecular weight of sample 1, as already hypothesized in the determination of SA.

CONCLUSIONS

The present study shows that direct infusion ESI mass spectrometry can be successfully used for quantifying concentrations of dicarboxylic acids in complex mixtures using the internal standard method. The concentration of terephthalic acid residue was determined using both the ESI-MS protocol and HPLC analysis with UV detection. The values obtained by the two methods are in excellent agreement. A low standard deviation for the concentration of sebacic acid was achieved. This assay represents a valuable alternative to conventional methods currently employed for acid quantification, resulting advantageous for its speediness and high sensitivity. It also may have great potential for quantitative analysis of other polymer components, such as oligomers, catalysts, and byproducts, whenever a suitable internal standard can be easily selected and, therefore, may represent a significant advance for the application of ESI-MS in polymer analysis.

ASSOCIATED CONTENT

Supporting Information. Calibration curve for terephthalic acid obtained by HPLC using the external standard method. This material is available free of charge via the Internet at http://pubs.acs.org.

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