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Automated Strong Cation Exchange Extraction of Fatty Acid Esters of 3-(*N*-Phenylamino)-1,2-propanediol from Oil Samples for Routine Quantification by HPLC-APCI/MS/MS

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Fatty acid esters of 3-(*N*-phenylamino)-1,2-propanediol are currently considered the best chemical markers of toxic oils related to the Spanish toxic oil syndrome. Recent research in this area has undertaken the exhaustive and quantitative characterization of these compounds in oils collected during the epidemic outbreak. Current methods developed in this laboratory are based on solid phase extraction (SPE) using SCX cartridges followed by HPLC-APCI/MS/MS quantification. To circumvent the long and tedious extraction procedure, the SPE protocol was adapted for automatic extraction and the problems derived from the use of the immiscible solvents required for the SCX extraction were solved. Linearity of the analytical method was found in the same range as for the manual method. Extraction recoveries were 87 and 75% for 2-hydroxy-3-(*N*-phenylamino)propyl linoleate and 2-(linoleyloxy)-3-(*N*-phenylamino)propyl linoleate, respectively, and the corresponding coefficients of variation were ~1%, greatly improving reproducibility over manual procedures.

Keywords: *Toxic oil syndrome; fatty acid esters of 3-(N-phenylamino)-1,2-propanediol; strong cation exchange; HPLC-APCI/MS/MS*

INTRODUCTION

The disease known as Spanish toxic oil syndrome appeared in Spain in 1981 (*1*) and affected >20000 people (*2*). Early epidemiological investigations related the toxic oil syndrome to the ingestion of cooking oil mixtures containing variable amounts of aniline (*1*). The origin of this chemical was a rapeseed oil denatured with 2% aniline in order to be imported from France for industrial use. Once in Spain, this oil was refined with the aim of removing the aniline, mixed with olive oil, and fraudulently marketed for human consumption.

Further studies showed that two families of aniline derivatives detected in toxic oils [fatty acid anilides and fatty acid esters of 3-(*N*-phenylamino)-1,2-propanediol) (**1**, Figure 1] (*3*) had a strong association with the toxic oil syndrome (*4*, *5*). These molecules were probably produced from the reaction between aniline and the fatty acids and triglycerides in oil.

Aniline derivatives are considered to be useful chemical markers of toxic oils. On the other hand, the esters of compound **1** have been suggested as potential etiologic agents of the disease (*5*). The interest in these compounds was renewed when they were related to the eosinophilia myalgia syndrome (*6*, *7*). This disease showed clinical similarities to the toxic oil syndrome, and it was demonstrated that compound **1** could be metabolically transformed into 3-(*N*-phenylamino)-L-

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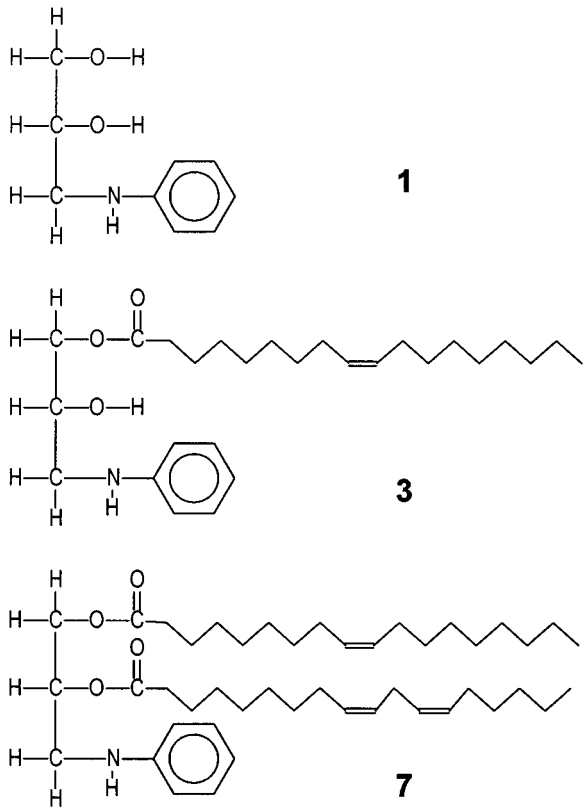

Compound	Chemical Name	(M+H)+
1	3-(*N*-phenylamino)-1,2 propanediol	168
2	2-Hydroxy-3-(*N*-phenylamino)propyl linoleate	430
3	2-Hydroxy-3-(*N*-phenylamino)propyl oleate	432
4	2-Hydroxy-3-(*N*-phenylamino)propylheptadecanoate	420
5	2-Linoleyloxy-3-(*N*-phenylamino)propyl linoleate	692
6	2-Oleyloxy-3-(*N*-phenylamino)propyl linoleate	694
7	2-Linoleyloxy-3-(*N*-phenylamino)propyl oleate	694
8	2-Oleyloxy-3-(*N*-phenylamino)propyl oleate	696
9	2-Oleyloxy-3-(*N*-phenylamino)propyl heptadecanoate	698

Figure 1. Compounds studied in this work and *m/z* values of their protonated ions.

alanine, a chemical contaminant related to the eosinophilia myalgia syndrome. However, neither anilides nor compound **1** derivatives have demonstrated specific toxicity related with the toxic oil syndrome. The lack of a suitable animal model that fully duplicates the syndrome has been and still is the main barrier for the identification of the etiologic agent(s) (*8*).

Currently, considerable analytical effort is being applied to achieve a thorough characterization of all toxic oil syndrome-related oil samples collected during the epidemic outbreak in order to determine the possible causes of the disease (*9*). Some methods for the analysis of the fatty acid esters of compound **1** based on the direct analysis of oil samples by HPLC-APCI/MS/MS have been proposed by groups involved in the toxic oil syndrome research (*4*, *10*). A solid phase extraction (SPE) method for the cleanup and preconcentration of these compounds in oil using strong anion exchange (SCX) cartridges has been developed for their analysis by HPLC-UV (Blount et al., unpublished results). The SCX extraction is highly selective for these compounds because not many of other basic components are expected to be present in edible oil. Recently, we optimized this SPE method for HPLC-APCI/MS/MS analysis, and the complete procedure was validated for quantification (*11*, *12*). More than 2600 oil samples were analyzed by using this method in our laboratory.

The main drawbacks of the analytical procedure were found in the sample preparation steps. The manual SPE step was labor- and time-intensive and requires skilled and attentive personnel to ensure constant recoveries. To circumvent these problems, we describe herein an automated procedure for sample cleanup based on this selective SCX extraction.

MATERIALS AND METHODS

Solvents. All solvents were of Lichrosolv grade and were obtained from Merck (Darmstadt, Germany). Acetic acid (96% pro-analysis) was also from Merck. Ammonium citrate was obtained from Sigma (Sigma-Aldrich, Madrid, Spain). Methylamine (40%) was from Scharlau (Barcelona, Spain). The scintillation liquid used for radioactivity measurements was Optiphase "Hisafe" from Wallac (Fisons Chemicals, U.K.). Milli-Q grade water used was on-line filtered with a Millipak-40 0.22 μm pore filter (Millipore, Mollsheim, France).

Compounds **1**−**9** as well as standards of compounds **2** and **5** with the aniline ring labeled with the radioisotope ^{14}C were provided by Dr. Angel Messeguer (CID,CSIC, Barcelona, Spain). Positional isomers **6** and **7** show the same chromatographic and mass spectrometric characteristics, and their differentiation requires the use of MS^3 procedures (*12*). Using our HPLC-APCI/MS/MS analysis the actual isomer composition cannot be determined. Therefore, they are referred to generically as compound **6** in the text.

Compounds **4** and **9** were used as internal standards for quantification (3.1 and 1.4 μg/mL in 2-propanol, respectively).

Toxic oil syndrome-related oil samples were provided by the CISAT (Centro de Investigación sobre el Síndrome del Aceite Tóxico, Madrid, Spain).

Solid Phase Extraction. *Manual Procedure.* Oil samples were diluted in 2-propanol at 200 mg/mL. Internal standards (100 μL) were then added to a 200 μL aliquot of the oil sample (40 mg of oil). This solution was diluted with 1 mL of ethyl acetate/hexane (1:1), vortex mixed, and loaded onto an SCX cartridge (SDS, Barcelona, Spain). The cartridge was washed with 9 mL of ethyl acetate/hexane (1:1), 3 mL of ethyl acetate, and 3 mL of methanol. Finally, 3 mL of ammonium citrate (tribasic, 0.1 M in water) was added to provide counterions for the subsequent elution. The column was eluted with 3 mL of toluene/2-propanol/acetonitrile (50:45:5), and the biphasic eluant was collected in a 4 mL polypropylene conical tube. Methylamine (200 μL) was added to the biphasic eluant to enhance recovery of the fatty acid esters of compound **1** in the organic solvent. Then the organic phase was isolated and evaporated close to dryness (5−10 μL remaining) in a Speed Vac concentrator. The residue was diluted with 350 μL of 2-propanol/methanol (1:1) and stored at −20 °C until analysis. During method development two different SCX cartridges from Varian (brand 1) and UCT (brand 2) were tested.

Automatic Method. The automatic procedure was carried out using a Spark Holland Marathon automatic injector connected to a Prospekt unit provided with a solvent delivery unit for automated SPE (Figure 2). Bondesil SCX cartridges from Spark Holland were used for SPE. For the analysis, internal standards (200 μL) were added to an 80 mg oil sample in 200 μL of 2-propanol, and the solution was diluted to a final volume of 1 mL with ethyl acetate/hexane (1:1).

The Prospekt unit used three valves as shown in Figure 2. Valve 1 (V1) was connected to the pump in the solvent delivery unit, to the injector, and to a secondary pump (Waters model M6000, Waters, Milford, MA); V2 was connected to the SCX cartridge holder; and V3 was connected to the fraction collector. The secondary pump connected to V1 was permanently pumping ammonium citrate (0.1% in water) at 1 mL/min.

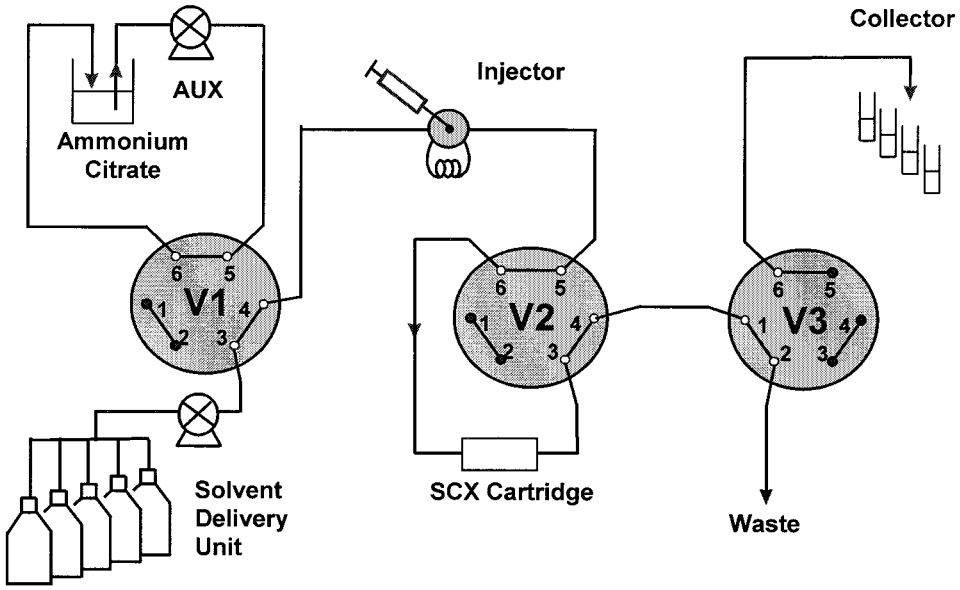

Figure 2. Scheme of the setup used in the automatic extraction procedure. V1−3 correspond to the switching valves provided in the Prospekt system. The AUX pump was an independent HPLC pump continuously flushing an ammonium citrate solution.

Table 1. Solvent and Valve Programs for Automatic Extraction of PAPs

time	solvent[a]	SDU flow rate, mL/min	injector	V1 citrate	V2 cartridge	V3 collector	comments
0	1	5	load	recycle	cartridge	waste	conditioning
1:00	2						
2:00	2	0.3	inject				sample load
6:00	3	5			bypass		tubing wash
6:30	3	1			cartridge		cartridge wash
7:00	4	5			bypass		tubing wash
7:30	4	0		input			tubing wash (citrate)
8:30					cartridge		citrate
9:30	4	5		recycle	bypass		tubing wash
10:00	5						
10:30	5	1			cartridge	collect	
12:30	4	5				waste	system wash
13:30	0	0					

[a] Solvents: 1, 2-propanol; 2, ethyl acetate/hexane; 3, ethyl acetate; 4, methanol; 5, toluene/2-propanol/acetonitrile.

Depending on the position of V1, citrate was either sent to the cartridge or recycled to its container in a closed circuit.

The solvent, flow rate, and valve programming are shown in Table 1. Once the cartridge was connected to V2, the first step was to equilibrate the cartridge with 2-propanol and ethyl acetate/hexane (1:1). Then, 200 μL of sample, previously diluted as indicated above, was injected into the system and passed through the SCX cartridge. After three consecutive washes with ethyl acetate/hexane (1:1), ethyl acetate, and methanol, the ammonium citrate solution was delivered by the auxiliary pump for 2 min. Finally, compounds were eluted with toluene/2-propanol/acetonitrile (50:45:5). During the elution time, V2 was switched to connect the system to the fraction collector. The collected eluate was evaporated close to dryness in a Speed Vac concentrator. The residue was diluted with 100 μL of 2-propanol/methanol (1:1) and stored at −20 °C until analysis.

Extraction Recovery. Compounds **2** and **5** labeled with ^{14}C were used as tracers for recovery calculations.

For manual SPE, 100 μL (100000 dpm) of the labeled standard solution was added to 50 mg of oil. The mixture was diluted with 1 mL of ethyl acetate/hexane (1:1) and then loaded onto a previously equilibrated SCX cartridge. The extraction procedure explained above was carried out, collecting all of the eluted material, including the washing steps, into scintillation tubes. The fractions collected were as follows: (1) nonretained material during sample loading; (2) three consecutive 3 mL ethyl acetate/hexane washes; (3) ethyl acetate wash (3 mL); (4) methanol wash (3 mL); (5) 3 mL of ammonium citrate; and (6, 7) two consecutive 3 mL elutions with toluene/2-propanol/acetonitrile.

The different fractions were evaporated with a helium stream and redissolved in 2-propanol (1 mL). Scintillation liquid (8 mL) was added to each tube and the radioactivity measured in a Wallac model 1410 beta counter.

For automatic extraction the same procedure was followed. In this case, 500 μL of the labeled standard was added to 100 mg of oil matrix. The mixture was diluted with 400 μL of ethyl acetate/hexane 1:1. After 200 μL of the solution had been injected to the system (22 mg of oil), the following fractions were collected: (1) nonretained material during sample loading and ethyl acetate/hexane wash; (2) ethyl acetate wash; (3) ammonium citrate solution; and (4) elution solution. These fractions were evaporated and redissolved with 1 mL of 2-propanol and 8 mL of scintillation liquid. Then the radioactivity was measured as before.

The radioactivity retained in the columns after the final elution step was also measured. For this purpose, the cartridges were cut and the sorbent was transferred to 50 mL polypropylene tubes (Falcon). The sorbent was then extracted with 15 mL of the SPE elution mixture (toluene/2-propanol/acetonitrile) in an ultrasonic bath for 15 min. The solid phase was removed by centrifugation, the organic extract evaporated in scintillation tubes, and the radioactivity measured as indicated before.

Calibration Curves. Calibration samples for manual extraction were prepared as follows: Aliquots of 40 mg of a standard oil matrix were spiked with eight different calibration

mixtures containing compounds **3**, **5**, **6**, and **8**. The oil selected as calibration matrix corresponds to an oil lacking any measurable level of the target compounds. Concentration levels in the calibration mixtures ranged from 0.2 to 100 ppm. Then, 100 μL of internal standard mixture (3.1 μg/mL of compound **4** and 1.4 μg/mL of compound **9**) and 1 mL of ethyl acetate/hexane (1:1) were added. The total volume was submitted to SPE extraction.

For the automatic method, aliquots of 80 mg of oil matrix were spiked with the corresponding standard calibration mixtures. To these samples were added 200 μL of the corresponding internal standard mixture and 400 μL of ethyl acetate/hexane (1:1) to obtain a total volume of 1 mL. An aliquot of 200 μL was loaded into the extraction system.

HPLC-APCI/MS/MS Analysis. Chromatography was carried out using a model 1100 HPLC from Hewlett-Packard with a binary pump and a UV detector. Samples were injected with a Triathlon automatic injector (Spark Holland B.V., Emmen, Holland) provided with a 50 μL loop.

The analytical column was a reversed phase Partisil ODS-3 (5 μm particle size, 150 × 2 mm i.d.) from Tecknokroma (Barcelona, Spain) preceded by a 10 × 2 mm i.d. ODS precolumn (Upchurch Scientific, Oak Harbor, WA). The injection volume was 10 μL and the flow rate 300 μL/min. Target compounds were separated under gradient conditions. Solvent A was composed of water/methanol (20:80) (0.1% acetic acid), and solvent B was 2-propanol/methanol (20:80) (0.1% acetic acid, 0.5% hexane). The gradient started at 20% of B and increased to 80% in 0.1 min, then increased again to 100% of B in 3.5 min, at which it was held for 5 min.

Mass spectrometric analysis of the column effluent was carried out by connecting the column to the atmospheric pressure chemical ionization (APCI) source of a TSQ 700 mass spectrometer (Finnigan, San Jose, CA). Source parameters were as follows: corona discharge, 1 kV; vaporizer temperature, 500 °C; heated capillary temperature and voltage, 250 °C and 28 V, respectively; tube lens voltage, 81 V; sheath gas (nitrogen) pressure, 20 psi; no auxiliary gas was used.

Quantitative measurements were achieved using two different internal standards: compound **4** for quantification of the monoesters and compound **9** for the diesters of compound **1**. Analyses were carried out in the precursor-ion scan mode. $[M + H]^+$ ions at *m/z* 432, 420, 684.5, 692.5, 694.5, and 696.5 (for compounds **3**, **4**, **9**, **5**, **6**, and **8,** respectively) were monitored in the first quadrupole and the common product ion at *m/z* 132 in the second analyzer (third quadrupole). The collision energy was set at −25 eV for monoesters and at −35 eV for diesters. The collision gas (argon) pressure was 1.5 mTorr, and the multiplier voltage was set at 1800 V.

RESULTS AND DISCUSSION

SCX Extraction. The SCX method used in this investigation takes advantage of the diverse interactions ocurring between the fatty acid esters of compound **1** and the SCX stationary phase to produce a highly selective extraction. Ionic interactions between the amino group from the target compounds and the SO_3^- from the sorbent determine their retention. Additionally, apolar interactions between the phenyl group on the esters of compound **1** and the sorbent also influence the extraction process. A careful solvent selection is thus important to maintain the equilibrium between both interactions necessary for high recovery.

The original SCX method was developed for HPLC-UV analysis and used 500 mg of oil for extraction. To adapt the procedure for HPLC-APCI/MS/MS analysis and to optimize recovery and reproducibility, only $^1/_{10}$ of this amount of oil was extracted in our procedure. Additionally, we suspected that a fraction of the target compounds could be lost as a citrate salt in the aqueous layer of the final eluate. These losses could be responsible for the high variability in the absolute peak areas observed in the analysis of samples spiked at the same concentration level. To prevent this phenomenon, an additional liquid−liquid extraction of the biphasic eluate was performed with the addition of 200 μL of a methylamine solution (1% in water). After vortexing and centrifugation, the aqueous phase was eliminated. With this treatment, reproducibility was greatly enhanced. Coefficients of variation obtained for the absolute areas of the internal standards **4** and **9** were 25 and 15% (n = 54), respectively. Before the treatment, these values were as high as 65 and 35% (n = 100) for the same standards.

An important point in the extraction procedure is to avoid cartridge drying after the ammonium citrate wash. In the original procedure the cartridge was dried to minimize aqueous ammonium citrate in the extract. This produced highly variable recoveries due to the difficulty in reproducing the same level of drying with different cartridges. In the present, optimized procedure, the esters are eluted with the organic solvent from the wet cartridge and the abundant aqueous phase collected with the organic eluant is then eliminated during the methylamine extraction procedure.

The complexity of the interactions involved in the SCX extraction suggested that important differences in recovery could be found between SCX solid phases of different composition. For this reason, two different brands of commercially available SCX columns were compared during method development. Recoveries were monitored by using oils spiked with the radiolabeled (^{14}C) compounds **2** and **5**.

Total recoveries for compounds **2** and **5** in the manual extraction were 66.7 and 79.3%, respectively (brand A column), and 79 and 85%, respectively (brand B column). During the extraction procedure, major losses were observed for **2** in the washes with polar solvents such as MeOH and ammonium citrate (collected fractions 4 and 5, respectively; see Materials and Methods). Compound **5** was well retained in the column during these steps. When using cartridges from brand A, an important fraction of both standards (3−5%) remained adsorbed after elution. This contributed to the lower recoveries obtained with these columns in comparison with the other cartridges (brand B). In general, cartridges from brand B were considered more appropriate for our specific procedure.

Automatic Extraction. The first protocols used for automatic extraction were designed to mimic the solvent order and flow rates used for manual extraction. These procedures soon proved to be inappropriate. The solvent mixing occurring when ammonium citrate solution was pumped after methanol caused ammonium citrate precipitation and the plugging of the solvent delivery unit solvent valves after a few cycles. This problem was solved by installing an additional, independent high-pressure pump. Thus, the main solvent delivery unit pump supplied organic solvents such as methanol, 2-propanol, ethyl acetate/hexane, and the toluene/acetonitrile/2-propanol elution mixture, and this second pump was responsible for ammonium citrate pumping. Valve 1 is commonly used in general protocols to allow a gas stream input (helium) for cartridge drying before elution. In our procedure, cartridge drying was not recommended and, therefore, this valve could be used

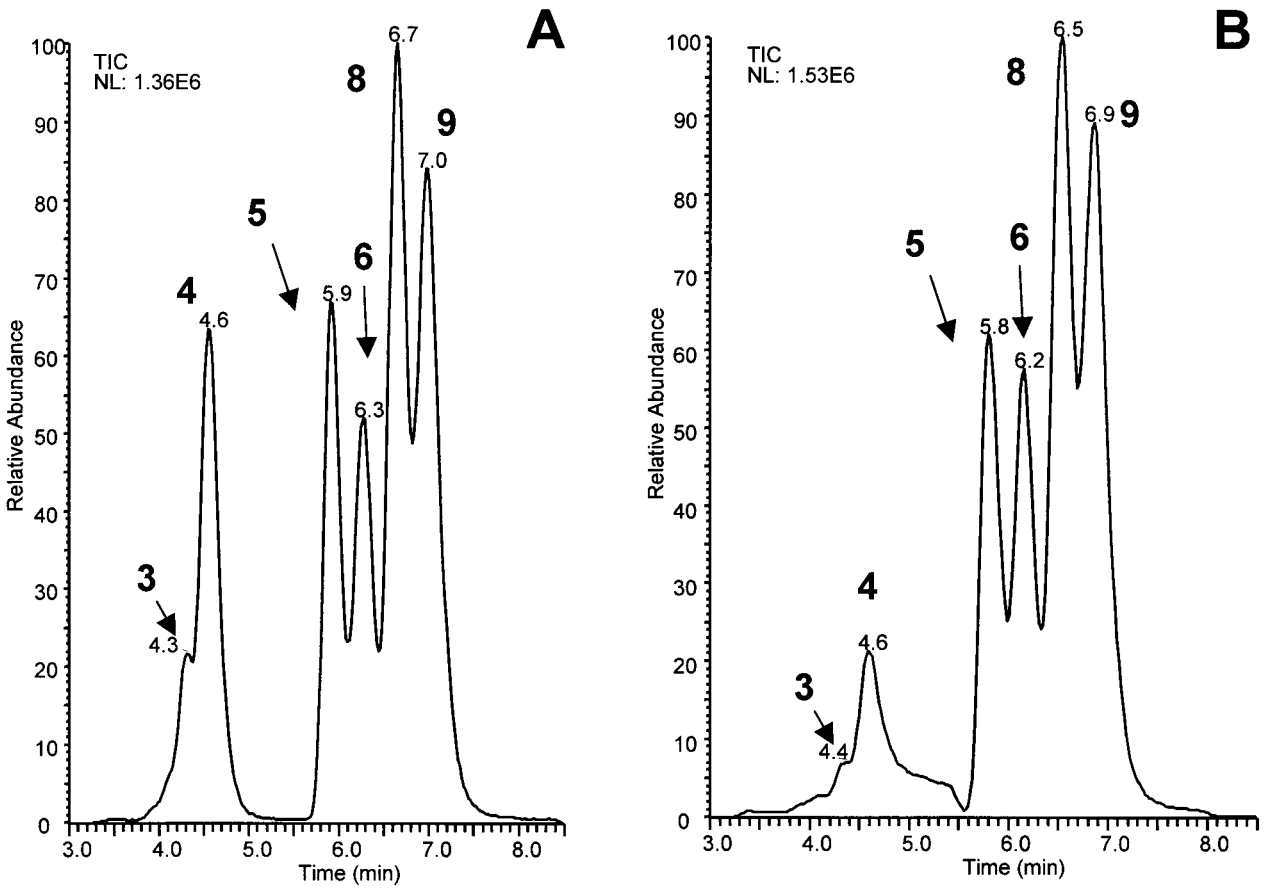

Figure 3. HPLC-APCI/MS/MS chromatograms obtained from an oil sample spiked with the esters **3** (2.5 ppm), **5** (2.9 ppm) **6** (2.6 ppm), and **8** (5.3 ppm) after (A) automatic and (B) manual extraction.

to switch solvent delivery between the solvent delivery unit solvents and ammonium citrate (see Figure 2 and Table 1).

As indicated above, major losses in the manual procedure were observed when methanol was used as intermediate solvent between the immiscible ethyl acetate and ammonium citrate solutions. Losses were observed both in the methanol fraction, mixed with some remanent ethyl acetate from the previous step, and in the citrate fraction, mixed with some methanol, and they were probably due to the elution effect produced by these mixtures of intermediate polarity. To prevent sample losses and, at the same time, to allow switching between immiscible solvents, the automatic system was programmed to pump methanol and ammonium citrate with the SPE cartridge disconnected from the flow path (V2 in bypass position). After the tubing was filled with ammonium citrate, this solution was pumped for 1 min through the cartridge. Then, V2 was switched to the bypass position and the system flushed with the apolar elution solvent before elution. The sequence of solvents pumped through the cartridge was then ethyl acetate−citrate−toluene/2-propanol/acetonitrile. This uncommon procedure produced a high recovery of the material in the column with minimal sample losses in the ammonium citrate fraction.

The initial oil dilution was also modified for the automatic mode. When the automatic method was used, only a fraction of the total sample volume could be injected into the extraction system. For practical purposes, the maximum injection volume was 200 μL. Oil dilution was calculated accordingly to inject the same sample weight compared with the manual method. In this respect, the volume of the sample loop on the injector was also shown to be critical for reproducibility and high recovery. Two hundred microliters of radiolabeled compound **5** was injected using a 200 or 500 μL sample loop. Immediately after injection, the eluent was collected into a scintillation tube at the exit of the injector. Radioactivity recovery was 78% when using the 200 μL loop and 97% with the 500 μL loop. The coefficients of variation also showed lower values with the 500 μL loop (2 versus 4%).

Although absolute recoveries in the automatic method were of the same magnitude as those obtained with the manual procedure (87 and 75% for **2** and **5**, respectively), the relative recovery of the monoester **2** versus the diester **5** was inverted. The high losses of compound **5** detected during sample loading (collected fraction 1; see Materials and Methods) seem to account for the observed differences. In this step, 2 and 17% of the total radiactivity from compounds **2** and **5**, respectively, were lost. Sample loading was performed using the same solvents in the manual and automated procedures; therefore, differences between methods should be explained by considering the different characteristics of the SPE cartridge. At the time the system was set up, there was no other SCX sorbent available from the supplier, and no further studies were carried out. Due to the general efficiency of the procedure developed, the availability of a sorbent with a higher retention potential than that currently tested should produce recoveries in the 95% range for these compounds.

A remarkable characteristic of the automatic procedure was its high reproducibility. Recoveries calculated from 10 independent samples showed coefficients of

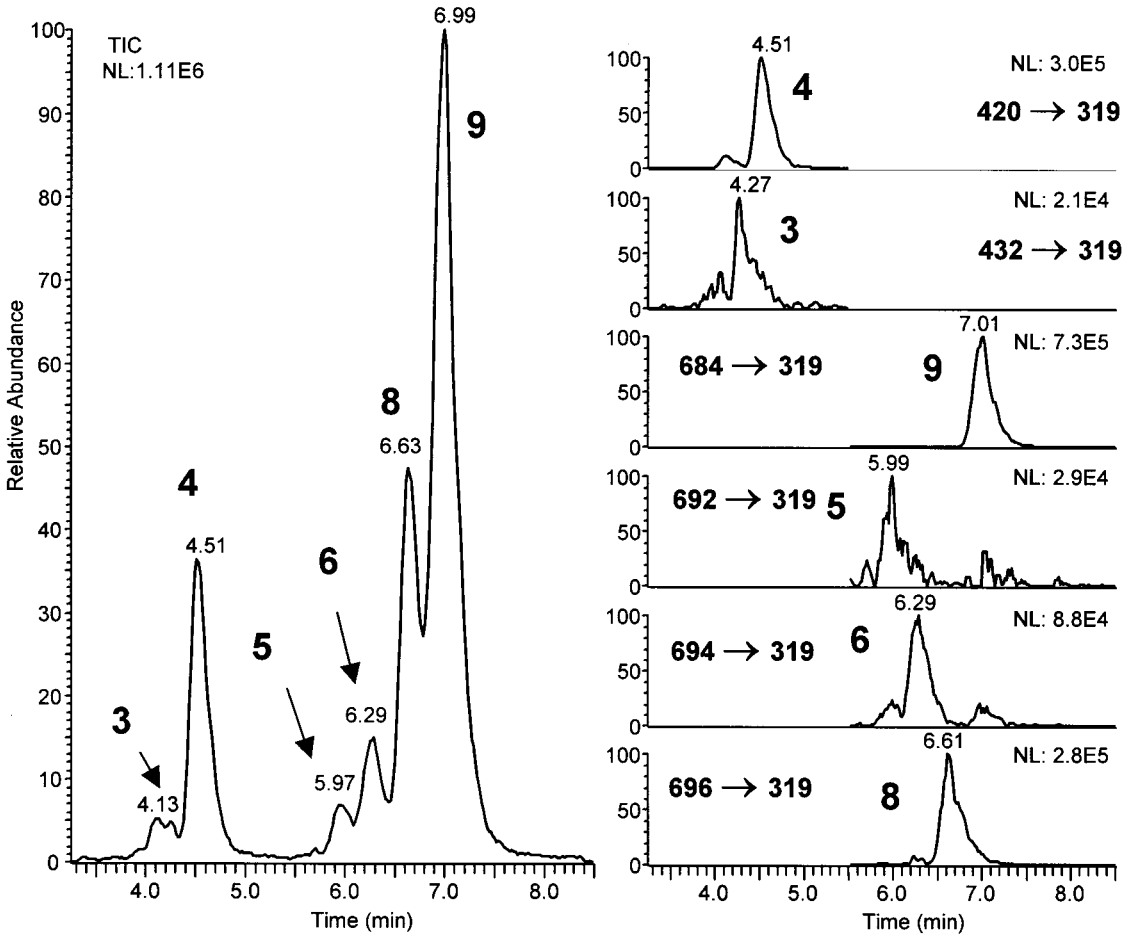

Figure 4. HPLC-APCI/MS/MS chromatogram (left) and extracted ion chromatograms (right) corresponding to the analysis of a toxic oil syndrome-related oil sample. The sample was prepared by automatic extraction and showed quantifiable amounts of 3-(*N*-phenylamino)-1,2-propanediols (2 ppm for compound **8**).

variation of 1.1 and 0.8% for radiolabeled compounds **2** and **5**, respectively. These values were between 2 and 8 times lower than those obtained with manual extraction.

HPLC-APCI/MS/MS Quantification. For routine quantification, a calibration curve for each target compound was performed daily using an oil matrix spiked with different levels of standards (oil 4078). The oil matrix was a toxic oil syndrome-related sample lacking any measurable level of fatty acid esters of compound **1**. Oil 4078 came from the same sources as oils containing aniline byproducts and was collected during the same time period. Therefore, it was considered to be an appropriate and representative matrix for calibration purposes.

The analytical characteristics of the HPLC-APCI/MS/MS method using the manual extraction procedure have been described elsewhere (*12*). During this comparative study and using the manual extraction, quantification limits, based on a signal-to-noise ratio of 10, were estimated at 0.2 ppm for the diesters and at 0.5 ppm for the monoesters. Similar quantification limits were obtained with the automatic extraction for mono- (0.4 ppm) and diesters (0.2 ppm). Calibration curves were linear throughout a calibration range from 0.2 to 100 ppm using either the manual or the automatic extraction. The coefficients of determination (r^2) were >0.999 in all cases.

Typical chromatograms obtained from manual and automatically extracted standard oil samples spiked with the target compounds are shown in Figure 3. Note that the relatively higher extraction efficiency of the automated method (Figure 3A) resulted in improved detection of the monoesters (eluting around 4.5 min) in comparison to the manual method (Figure 3B). The complete HPLC-APCI/MS/MS analysis was carried out in <10 min. Samples were injected every 11 min, approximately the same throughput as the automatic extraction step.

The method developed here was applied to the analysis of toxic oil syndrome-related oil samples obtained from CISAT storage (Madrid). These oils did not show a homogeneous composition, due to their different original sources. Often, they can be classified by their different odors, colors, and viscosities. Despite this heterogeneity, no interferences were found during sample analysis as a result of the high selectivity of the extraction procedure and the MS/MS analysis. Figure 4 shows a chromatogram corresponding to the analysis of an oil sample previously extracted with the automated procedure. The level of compound **8** in this sample was calculated to be 2 ppm.

Analysis of Low-Concentration Samples. As expected from the recovery data, the automatic extraction method yielded more sensitive detection for the monoesters than the manual extraction procedure. Oil samples that showed concentrations of compound **8** in the range from 1 ppm to below the quantification limit (0.2 ppm) were analyzed using the automatic procedure. It was shown that compound **3**, usually less abundant

than compound **8** and not detectable in any of these samples when using the manual procedure, was still detectable in 4% of the oils after automated extraction. Less clear differences between methods were observed for the analysis of the diesters **5**, **6**, and **8**. In some samples (14%) these compounds were detected only in the automated mode, whereas in other samples (10%) they were detected only when using the manual procedure. Despite the higher recovery obtained for diesters with the manual extraction, its lower reproducibility would explain these contradictory results with low-concentration samples.

Cleanup procedures are thus crucial for the analysis of fatty acid esters of compound **1** at low levels in oil matrices. Absolute recoveries of automated SCX SPE are similar to those obtained by manual extraction. Despite that, automation offers important advantages over manual procedures in terms of time and personnel required. Moreover, automated SPE minimizes human intervention and optimizes analysis robustness and reproducibility. The selective SPE procedure reported herein for the analysis of 3-(*N*-phenylamino)-1,2-propanediol derivatives is currently used in our laboratory for the extraction of other apolar aniline derivatives and could be extended to any other amine provided the basic amino group is sterically accessible.

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