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Confirmation of multiple tetracycline residues in milk and oxytetracycline in shrimp by liquid chromatography–particle beam mass spectrometry

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Abstract

A confirmation procedure is described for detection of residues of six tetracyclines in bovine milk, and oxytetracycline in shrimp. Residues are extracted from milk or shrimp tissue using metal chelate affinity chromatography. The extracts are desalted, further concentrated using polymeric solid-phase extraction, and chromatographed on a polymeric reversed-phase column. Analysis is by methane negative ion chemical ionization on a quadrupole mass spectrometer using a particle beam interface. Data are acquired in partial scan mode, monitoring from m/z 378 to m/z 480. The procedure was validated with control milk and shrimp, fortified milk (30 ng/ml) and shrimp (100 ng/g), and milk and tissue from animals treated with the drugs. Published by Elsevier Science B.V.

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1. Introduction

Tetracyclines (TCCs; refers to drug class) are widely used in veterinary practice to control disease and promote growth. In the US, oxytetracycline (OTC) and chlortetracycline (CTC) are approved for use in lactating dairy cattle. Tetracycline (TC; refers to specific drug) is approved for use in veal and beef cattle. Other TCCs, such as minocycline (Mino), demeclocycline (DMC) and doxycycline (Doxy) are not currently approved for animal use, but may be available commercially. The US Food and Drug Administration (FDA) has set safe levels for residues of TC, OTC, and CTC in milk of 80, 30, and 30 ppb, respectively.

OTC is widely believed to be one of the most common antibiotics used in shrimp aquaculture, particularly in countries other than the US. Aquaculture production is important in the shrimp industry, where the percentage of total worldwide shrimp consumption produced by farming increased from less than 2% in 1980 to more than 26% in 1989 [1]. OTC is currently not approved for use in shrimp aquaculture in the US OTC medicated feed is approved for catfish, salmon, and lobster aquaculture [2]. The tolerance for OTC residues in edible tissues in these species is 2.0 ppm [3]. Under an Inves-

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tigational New Animal Drug authorization from the US FDA, Freleir et al. used OTC medicated feed to treat a cultured shrimp bacterial disease, necrotizing hepatopancreatitis [4]. OTC has also been used to treat vibriosis, one of the major bacterial diseases of penaeid shrimp [5].

Failure to discard milk for an appropriate time following lactating cow treatment or failure to ensure that the drug has depleted from shrimp tissue before harvest could result in residues in human food. Prior to the FDA taking regulatory action, the presence of suspected violative residues is usually confirmed using an assay of high specificity. Mass spectral analysis provides the high degree of specificity that is lacking in most liquid chromatographic (LC) procedures with UV or fluorescence detection.

This laboratory and others have previously published work on the confirmation of TCC residues in milk or tissue by mass spectrometry. Oka and coworkers employed thin-layer chromatography followed by fast atom bombardment (FAB) MS for the confirmation of OTC, TC, CTC and Doxy residues in bovine tissues [6] and milk [7]. The limit of detection (LOD) of this method, 50 ppb, was above FDA's safe level for OTC and CTC in milk. In addition, for some of the TCCs only the protonated molecular ion was detected by FAB-MS. For regulatory purposes, the FDA prefers that at least two diagnostic fragment ions be present to provide sufficient specificity of residue identification [8]. Oka et al. have recently reported an improved confirmation of these four TTCs in tissue using electrospray LC–MS–MS on a triple quadrupole instrument [9]. They validated the procedure at 0.1 ppm. The major limitation of the LC–MS–MS analysis was that only fragments resulting from loss of water and ammonia were observed in the daughter ion scan for three of the TTCs. Only loss of water was observed for doxycycline, which limited the specificity of the assay.

Blanchflower et al. recently described determination and confirmation of TC, OTC and CTC in tissue using atmospheric pressure chemical ionization (APCI) LC–MS [10]. For confirmation purposes, the instrument was run in selected ion monitoring (SIM) mode, looking at the protonated molecular ion, the ion resulting from loss of water, and the ion resulting from loss of ammonia (OTC and TC) or the chlorine isotope ion (CTC). Although the estimated LOD was low (0.01 to 0.02 μg/g, depending on tissue), the variability of fragment ion relative abundances was higher than desirable for SIM analysis [8].

Voyksner et al. [11] showed the utility of liquid chromatography–particle beam-mass spectrometry (LC–PB-MS) for the analysis of CTC and TC. By methane negative ion chemical ionization (NICI), they were able to observe both the molecular ion and multiple fragment ions (resulting from dehydration or other loss of functional groups) during analyses of standards. Kijak et al. [12] used this approach for the confirmation of OTC, TC and CTC residues in milk. Using SIM, they were able to monitor the molecular ion and three fragment ions for each residue from milk extracts prepared by acid protein precipitation, ultrafiltration, and silica C_{18} solid-phase extraction (SPE). The procedure was limited by insufficient sensitivity (the LOD was 100 ppb in milk), inconsistent recovery of TCCs from silica-based C_{18} SPE cartridges, and >10% difference between TCC standards' and TCCs in milk extracts' fragment ion relative abundances.

The procedure described here can confirm the presence of OTC in shrimp at 100 ng/g or OTC, TC, CTC, DMC, Doxy and Mino in raw bovine milk at a level of 30 ng/ml (ppb). The confirmation employed the LC–PB-MS approach used previously in this laboratory [12], but with a more efficient extraction procedure, different chromatography, and an updated mass spectrometer (MS) and PB interface. Metal chelate affinity chromatography (MCAC) [13–16] was used as an extraction and concentration step, followed by desalting and further concentration using polymeric SPE cartridges. The identities of TCCs extracted from milk were confirmed using LC–PB-MS. The residues were partially resolved from one another by separation on a polymeric reversed-phase column. The parent compounds were ionized using methane NICI. Regulatory specificity was achieved by scanning from m/z 378 to m/z 483. Confirmation was based on comparison of mass scans acquired from extracts with those acquired from individually injected standards. The sensitivity, specificity, and number of residues detected by this procedure are improvements on previously published mass spectral residue confirmation procedures.

2. Experimental

2.1. Reagents and chemicals

House distilled water was deionized and further purified by UV irradiation (OrganicPure, Barnstead, Dubuque, IA, USA). Methanol was HPLC grade (Burdick&Jackson, Muskegan, MI, USA). Reference standards of all TCCs were obtained from US Pharmacopeia Convention (Rockville, MD, USA). All other chemicals were reagent grade or better. Methanolic stock solutions of individual TCCs were prepared at 0.1 mg/ml and stored at −20°C. Fortified milk samples (30 or 80 ng/ml) were prepared by adding 12 or 32 μl (1200 or 3200 ng) stock drug solution to 40 ml raw control milk. Fortified shrimp samples were prepared by adding 10 μl stock solution to 10 g tissue. External standards for MS analysis were prepared by placing 0.1 ml stock solution in an autosampler vial, evaporating the MeOH under a stream of nitrogen, and reconstituting the residue in 1 ml water.

2.2. Animal treatment

Control milk was obtained from an individual cow known to be free of drug treatment. Milk samples containing incurred residues of individual TCCs were produced by orally dosing a cow with 2.5–10 mg/lb body mass of the drug. Milk was stored at −60 to −80°C prior to analysis.

Control black tiger shrimp (*Penaeus monodon*) were purchased from a local retail store. Ecuadoran white shrimp (*Panaeus vanamei*) were dosed with OTC at the University of Arizona under a contract with the FDA. Shrimp were fed medicated OTC feed at 1500 mg OTC/kg prior to harvest. This produced a very high level of residue incursion (~9.5 ppm). Dosed shrimp were composited with control shrimp using the dry ice grinding procedure described below to produce ground shrimp tissue containing near 100 ppb incurred OTC residue.

2.3. Shrimp tissue preparation

Two techniques were used to grind shrimp. In the first technique, used on the black tiger shrimp, shells, fins and tail were removed, and the meat was ground twice in a conventional meat grinder. The ground shrimp was mixed to ensure homogeneity and stored at −60°C until analysis.

In the second technique, Ecuadoran white shrimp (decapitated, but including shell, tail and midline) were ground using the dry ice procedure described by Bunch et al. [17]. Briefly, slab or pellet dry ice (150–200% by mass of the shrimp sample mass) was ground to a fine powder in a heavy duty vertical cutter mixer. Headless, frozen shrimp were added in portions to the dry ice and ground until a uniform powder was achieved. The powder was placed in a plastic container and put in a freezer loosely covered to allow the carbon dioxide to sublime. The ground shrimp tissue was then shipped on dry ice from Seattle to Beltsville and stored at −80°C until analysis.

2.4. Extraction and clean-up

Negative and positive (fortified) control samples were prepared with each sample set. Milk samples (40 ml) were placed in 50 ml disposable polypropylene centrifuge tubes and centrifuged at 2000 *g* for 15 min at 10°C to separate the cream. The lower 'skim' layer of the samples was transferred into clean tubes containing 2 ml glacial acetic acid. After mixing, the acidified milk was centrifuged at 2000 *g* for 25 min to pellet the resulting precipitate. Frozen ground shrimp tissue was partially thawed, 10 g weighed into 50-ml tubes, and homogenized with 0.1 *M* succinic acid, pH 4 (20 or 30 ml) until an even suspension was achieved. The homogenates were centrifuged at 2000 *g* for 15–20 min at 10°C.

Metal chelate affinity columns were prepared by swirling Chelating Sepharose Fast Flow (Pharmacia cat. no. 17-0575-01, Piscataway, NJ, USA) in its bottle until an even suspension was obtained. The slurry was poured into graduated disposable polypropylene columns (Bio-Rad Econo-Pac columns, cat. no. 732-1010, Rockville Centre, NY, USA) until the packed resin reached a bed volume of 5 ml. After installing top frits, the columns were washed with 2–3 aliquots of water (10 ml), then 'charged' with 0.1 *M* $CuSO_4$ (1.5 ml). The blue columns were washed with an additional 10 ml water before use. Columns with exceptionally slow flow-rates were assisted by suction applied at the bottom.

Milk or shrimp tissue extract supernatant solutions were applied to the blue MCAC columns. The columns were washed sequentially with 5 ml 0.5 *M* NaCl, 10 ml water, 10 ml methanol and 10 ml water. Gravity feed only was used for the next two steps. McIlvaine buffer containing 0.5 *M* NaCl and 0.1 *M* Na_2EDTA [15] (3 ml) was added to the columns. The clear effluent was discarded. The TCCs were eluted from the columns into receiving tubes with an additional 8 ml McIlvaine–EDTA–NaCl buffer.

The extracts were desalted and further concentrated using 6 ml Supelclean™ ENVI™-Chrom P SPE cartridges (Supelco cat. no. 67225, Bellefonte, PA, USA) that had been conditioned with 2 ml methanol followed by 5 ml water. The blue MCAC eluates were applied under vacuum to the SPE cartridges. The MCAC eluate receiving tubes were rinsed with 2.5 ml water, and the rinse was added to the SPE cartridges which were then washed with an additional 2.5 ml water. They appeared white at this point, with no trace of blue remaining. They were dried by drawing air through them for 2–3 min. The TCCs were eluted into 15 ml conical glass tubes with approximately 5 ml methanol using gravity flow. The remaining methanol was removed from the SPE cartridges by applying vacuum. The SPE eluates were evaporated to dryness under a nitrogen stream at 45–55°C and reconstituted in 1 ml water. Extracts not analyzed immediately were stored refrigerated.

2.5. LC system

A Hewlett-Packard Series 1050 pump, equipped with helium sparged solvent reservoirs and either a manual injector with a 200-μl loop or a 1050 autosampler, was used for the analyses. The TCC residues were partially resolved from each other by separation on a PLRP-S, 150×3.9 mm, 5 μm particle size, 100 Å pore size column (Polymer Labs cat. no. 1111-3500, Amherst, MA USA) using a mobile phase of methanol–5 m*M* aqueous oxalic acid (58:42, v/v) at a flow-rate of 0.5 ml/min. For both external standards and extracts, 100 μl was injected. This was equivalent to 1 μg of external standard, but less than 0.1 μg for milk (30 ng/ml fortification) and shrimp (100 ng/g) extracts. To avoid premature skimmer cone clogging, a Rheodyne model 7000 diverting valve was installed between the column and particle beam interface. Column effluent was diverted during column equilibration and during prolonged breaks (>30 min) between injections. Runtime was 16 min or less, depending on the TCCs being analyzed.

2.6. Mass spectrometer conditions

MS detection was accomplished on a Hewlett-Packard 5989A mass spectrometer equipped with NICI capability, high energy dynode (HED) detector, and channel electron multiplier (K&M Electronics, Springfield, MA, USA). The LC was interfaced to the mass spectrometer with a Hewlett-Packard 59980B particle beam interface set at 60°C desolvation temperature and helium sheath pressure 40–45 p.s.i. (1 p.s.i.=6894.76 Pa).

The mass spectrometer source temperature and GC interface temperature were set at 250°C and the quadrupole temperature at 100°C. NICI was used with methane reagent gas at 1 torr source pressure. The instrument was calibrated and manually tuned using perfluorotributylamine (PFTBA), with the source voltages adjusted to maximize on fragment ions at m/z 452 and 414. The instrument was tuned so peak widths were 0.5 a.m.u. at half-height. The electron multiplier voltage was adjusted so parent ion response at m/z 633 was between 80 000 and 200 000 counts. The HED voltage was tuned according to the manufacturer's instructions.

For sample data acquisition the electron multiplier voltage was set at 200 V above the value obtained during manual tune. The scan mode acquisition range was m/z 378 to 483 with a scan rate of 0.6 scans/s. Acquisition time was approximately 16 min (10 min if not analyzing for doxycycline). Single drug external standards were injected within a few runs before or after analysis of extracts.

Data were collected and analyzed using Hewlett-Packard Vectra MS DOS CHEMSTATION, Version C.00.07 software. Spectra from the retention time of the residue were averaged and an averaged spectrum from an adjacent background area, usually at the start or end of the residue peak, was subtracted. Daily libraries were compiled from mass spectra of standards. A probability-based matching library search algorithm (Hewlett-Packard) was used to

screen extracted residue mass spectra for potential TCC identification.

2.7. Confirmation criteria

Three criteria had to be met for residue presence to be confirmed in a sample extract: (1) the retention time in the sample had to be within 3% of the residue's retention time in a contemporaneous standard; (2) the spectrum obtained from the putative residue peak visually matched the spectrum obtained from a contemporaneous standard. Since particle beam introduction with NICI provided a very rich and detailed spectrum for TTCs, no additional quantitative ion relative abundance criteria were needed; (3) the positive and negative quality control samples analyzed with the sample set confirmed and failed to confirm, respectively.

3. Results and discussion

3.1. Sample extraction

The MCAC extraction described by Carson [15] was readily scaled up from 5 ml to 40 ml milk to accommodate the higher limits of detection of the mass spectrometer relative to the UV detector. Unfortunately, using a gradient similar to that used in the determinative procedure to accomplish the final concentration/desalting steps proved highly impractical. Even when the initial column effluent was diverted, the high percentage of water remaining in the effluent caused significant reduction in PB-MS sensitivity. The XAD-2 desalting step used by Farrington et al. [14] was effective, but cumbersome and solvent consuming. Previous work using silica-based SPE cartridges indicated that addition of oxalic acid to the methanol eluent was essential for good recoveries of TCCs [6,12]. In order to avoid adding nonvolatile salts to the elution solvent, we evaluated 12 different SPE cartridges for TCC recovery, including some recently introduced cartridges containing polymeric media. The recoveries of each TCC from each SPE ranged from 0 to 1% for all six TCCs (using a graphitized carbon cartridge) to >80% for most of the residues [18]. Two of the polymeric cartridges, ENVI-Chrom P and Alltech's IC-RP, had clearly superior recoveries, surpassing the performance of all of the silica-based cartridges. ENVI-Chrom P was adopted for use in the confirmation extraction procedure.

The clean-up, desalting and concentration processes developed for milk were readily adapted to shrimp extracts. The absolute recovery for the various TCCs using this extraction from either milk or shrimp was near 50% as judged by chromatography with UV detection.

3.2. Particle beam NICI LC–MS analysis

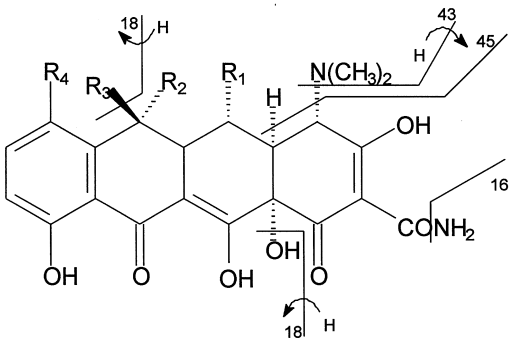
The LC–PB-MS analytical conditions were based on previous work done in this laboratory [12]. Other mobile phases containing more volatile buffers (such as trifluoroacetic or formic acid) were also evaluated and found to give adequate or even better resolution of TCC standards using the PLRP-S column. However, LODs for TCCs were markedly higher using these buffers than when a buffer containing the less volatile oxalic acid was used. This enhancement of signal by using a slightly nonvolatile buffer as a carrier was also reported by Kim et al. [19] and Mattina [20]. Increasing the aqueous content of the mobile phase generally resulted in decreased MS sensitivity. A diverter valve was installed between the LC and PB interface to minimize oxalic acid buildup on the skimmer cones. Skimmer cone clogging was much lower with the MCAC extracts than had been observed previously in this laboratory with cruder extracts [12], so routine use of the diverting valve during LC runs was not necessary.

Residue retention times were generally consistent, though not quite as reproducible as found in [15], between standards, fortified samples, and samples containing incurred residues. Within a set of consecutive analyses, retention times usually varied less than 0.1 min for the early eluting compounds (OTC and TC) and less than 0.4 min for Doxy, the latest eluter. Milk extract analyses were occasionally found to slightly change chromatographic performance. Injecting one or two negative control samples at the start of a set minimized this effect. To further ensure adequate retention time matching, sample analyses were usually immediately preceded and followed with standard analyses. Milk from treated animals sometimes exhibited peaks in addition to the parent

drug peak, but residue confirmation was based on analysis of the spectra at the retention time (t_R) of the parent drug.

Fig. 1 shows the structure of the TCCs studied and a proposed fragmentation consistent with the ions observed in methane NICI. The TCCs typically produced ion clusters at the molecular ion (M^-) and fragments resulting from the loss of one or two waters ($[M-18]^-$ and $[M-36]^-$), loss of dimethylamine (or carbamide) ($[M-44/43]^-$), loss of an amine or amide ($[M-16]^-$), or some combination of the above. Relative abundances, and even ions produced, varied with both mobile phase and instrument conditions.

All six TCCs produced a strong ion current for the molecular ion, but in only two cases, OTC and DMC, was the molecular ion the base peak. For TC and Mino, the fragment ion resulting from monodehydration was the base peak, for CTC loss of two water molecules produced the base peak, and for Doxy, m/z 382, resulting from multiple functional group losses, was the base peak. The relative intensities of some fragment ions varied between standards and sample extracts. This was especially true for the reaction producing the monodehydrated fragment ion.



Drug	R_1	R_2	R_3	R_4	m
Chlortetracycline	H	CH_3	OH	Cl	478
Demeclocycline	H	H	OH	Cl	464
Doxycycline	OH	CH_3	H	H	444
Minocycline	H	H	H	$N(CH_3)_2$	457
Oxytetracycline	OH	CH_3	OH	H	460
Tetracycline	H	CH_3	OH	H	444

Fig. 1. Structure of tetracyclines and their proposed fragmentation in LC–methane NICI PB-MS analysis.

There was also some shift in relative abundances observed over the course of several injections, particularly at the beginning of a set of analyses. This may have been due to buildup on the skimmer cones causing extra material to enter the source and thus affect ionization and fragmentation. A similar phenomena was reported during ivermectin residue analyses by Heller and Schenck [21].

Confirmation was initially done using SIM mode. Confirmation criteria included matching relative ion abundances to that of the standard within 10% for the three to six ions selected for each TCC. When analytical conditions were carefully controlled, the SIM acquisition mode almost always provided successful confirmation of TCC residues in milk extracts. However, due to the potential variability of the fragment ion relative abundances, it was uncertain whether this procedure was rugged enough to readily transfer to other particle beam instruments. Therefore, the use of partial scan acquisition (m/z 378 to 483) was also evaluated for confirmation of residues in milk extracts.

Since scan acquisition yields more detailed spectra, generating greater specificity, it is a preferred mode for confirmation analysis [8]. A major drawback in the past was that scan monitoring by LC–PB-MS was not sensitive enough for TCC residue analysis. The extraction process described in this paper provided a nearly 40-fold or 10-fold enrichment of the TCCs in milk or shrimp extracts, respectively, while removing most interferences. This extraction made scan mode analysis possible.

Fig. 2 shows a typical scan confirmation of OTC in milk fortified at 30 ppb. Panel a shows the TIC of the milk extract, with t_R of OTC at 5 min. Panel b is the spectrum obtained from the milk extract, and panel c shows the spectrum of OTC standard. All six residues were successfully confirmed at 30 ppb (and also at 80 ppb in the case of TC) in extracts of fortified milk samples using scan mode. Extracts of milk from dosed cows were also analyzed by mass scanning. The typical TICs and spectra for standards and extracts are in Figs. 3–8 and show the high quality of matches obtainable with this procedure. There were no interferences present in control milk. Control, fortified and incurred milk scan analyses

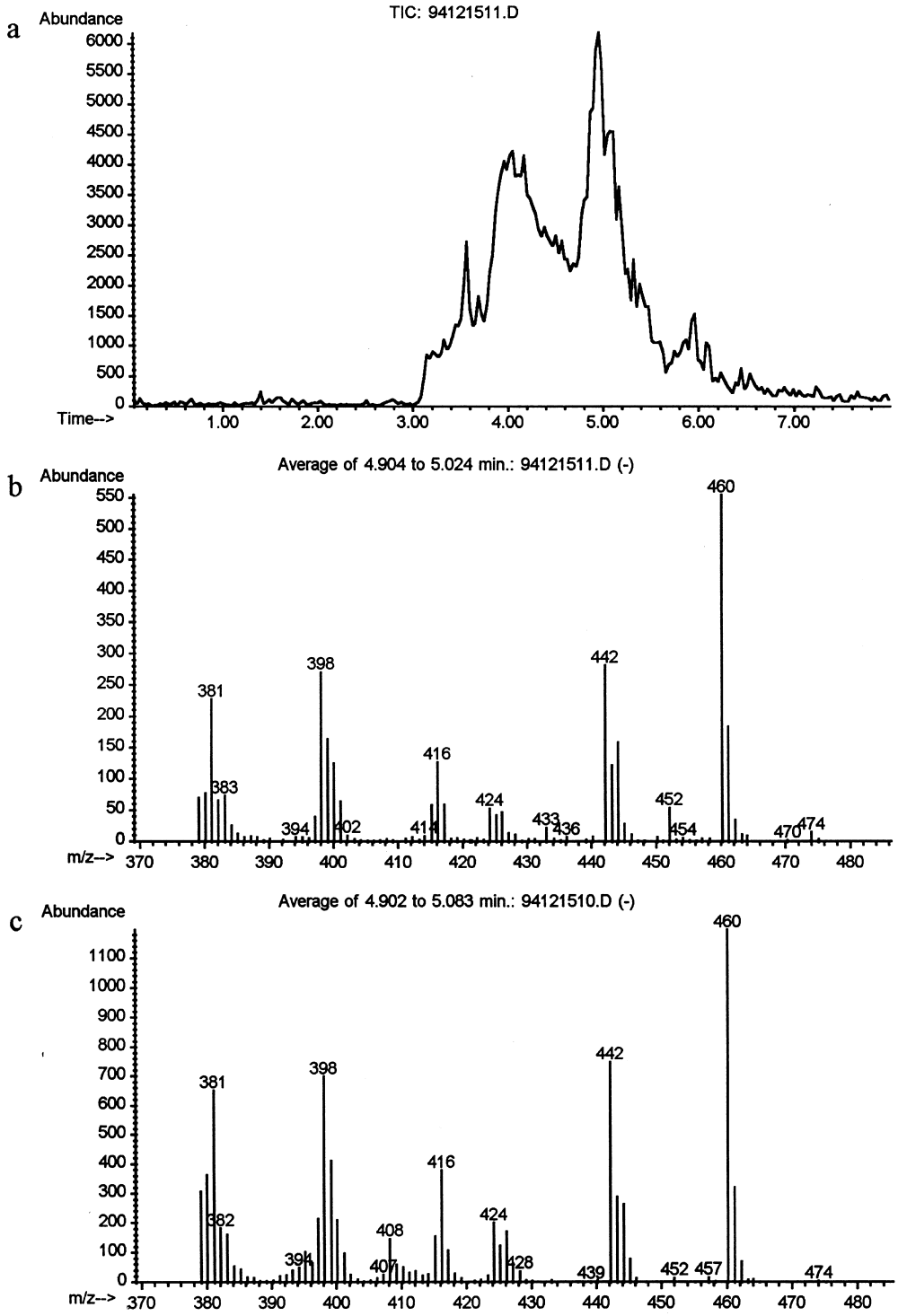


Fig. 2. Confirmation analysis by mass scanning of 30 ppb OTC fortified milk sample. (a) TIC of milk extract injection (100 μl); (b) averaged and background-subtracted spectrum from milk extract at OTC retention time; (c) spectrum of injected OTC standard (1 μg on column).

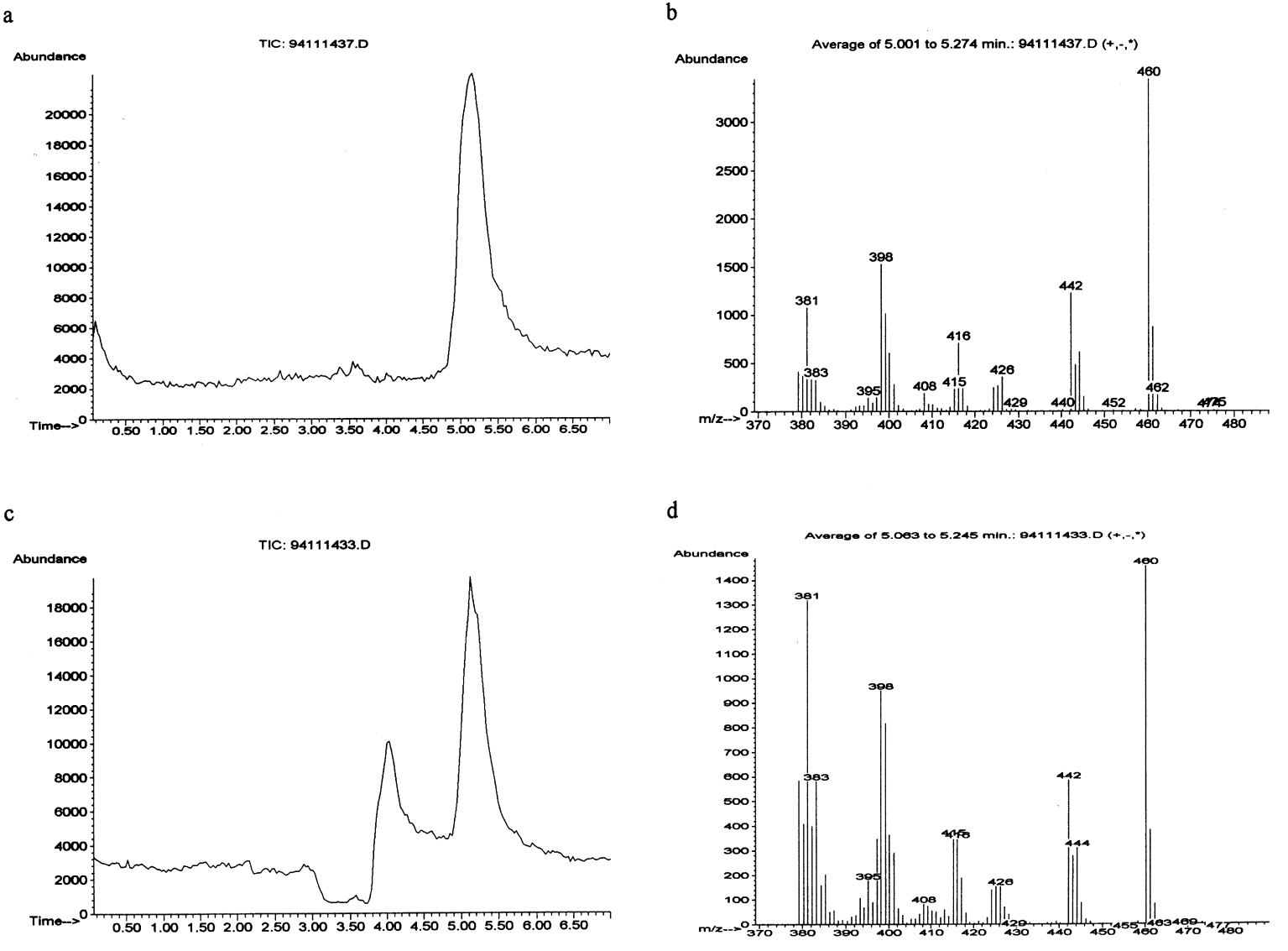


Fig. 3. Scan confirmation of incurred OTC. (a) TIC of standard; (b) NICI mass spectrum of OTC standard; (c) TIC of incurred OTC (24 ppb by determinative procedure analysis) milk extract; (d) spectrum of incurred OTC.

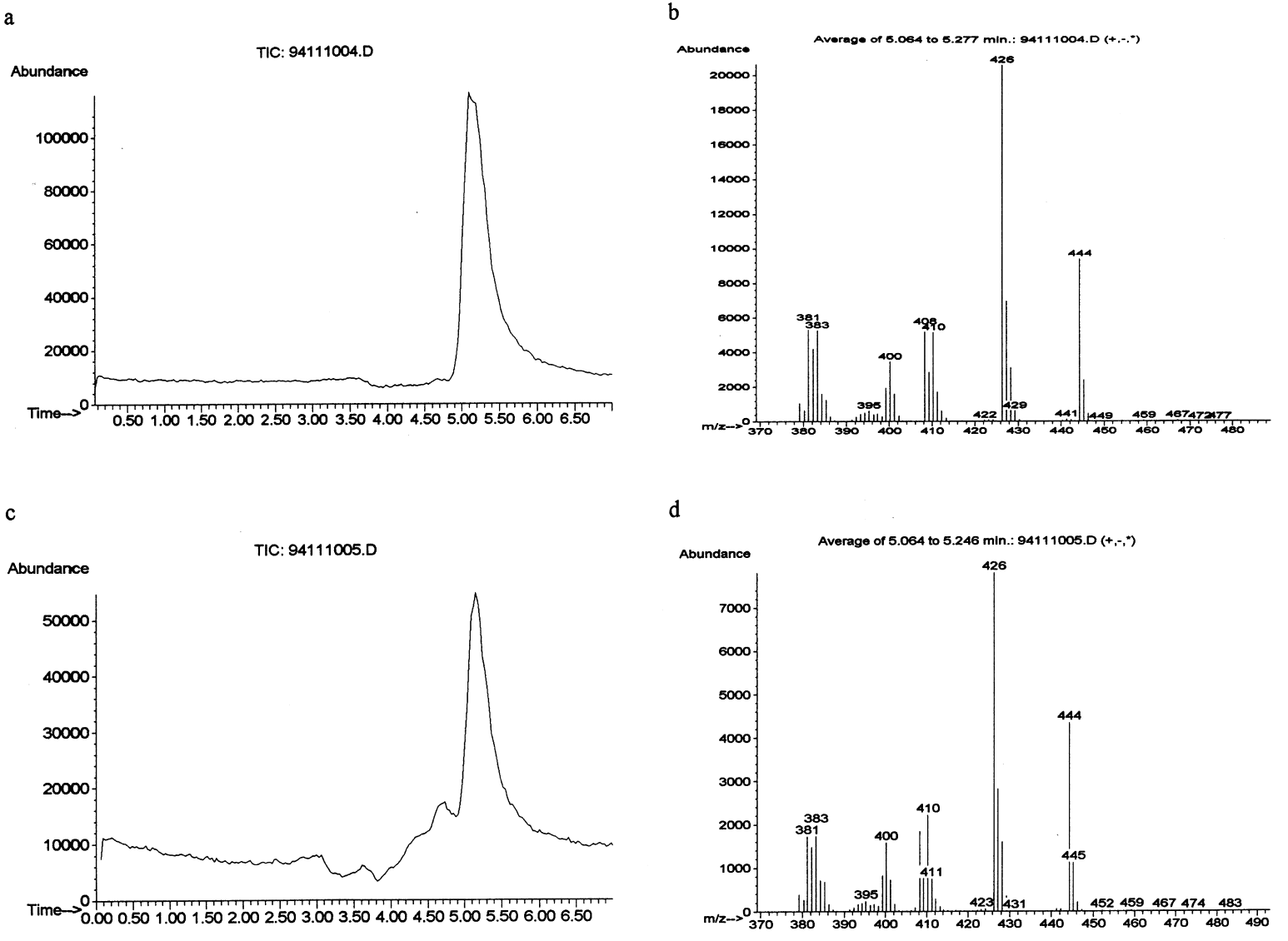


Fig. 4. Scan confirmation of incurred TC. (a) TIC of standard; (b) NICI mass spectrum of TC standard; (c) TIC of incurred TC (119 ppb by determinative procedure analysis) milk extract; (d) Spectrum of incurred TC.

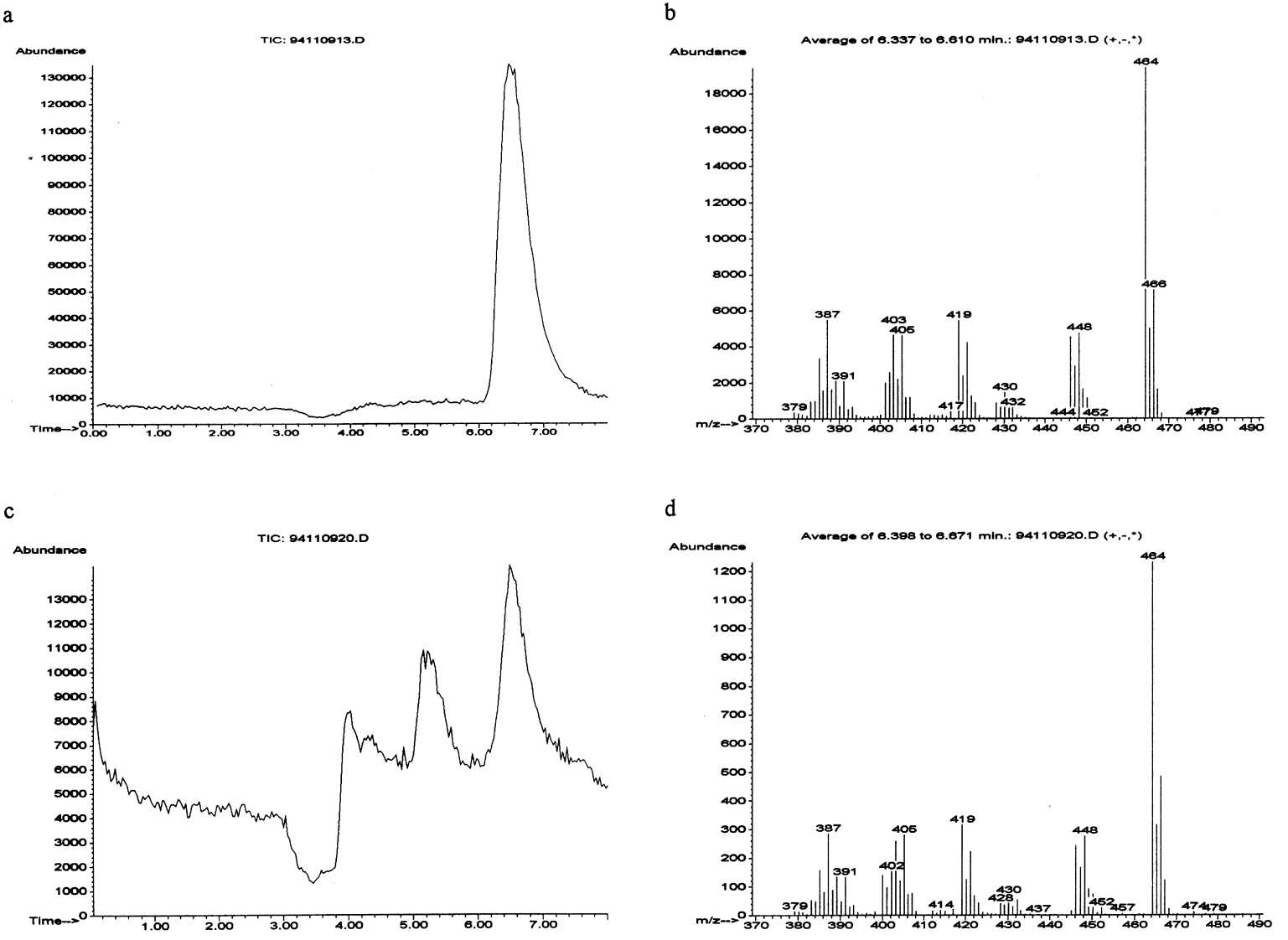


Fig. 5. Scan confirmation of incurred DMC. (a) TIC of standard; (b) NICI mass spectrum of DMC standard; (c) TIC of incurred DMC (35 ppb by determinative procedure analysis) milk extract; (d) Spectrum of incurred DMC.

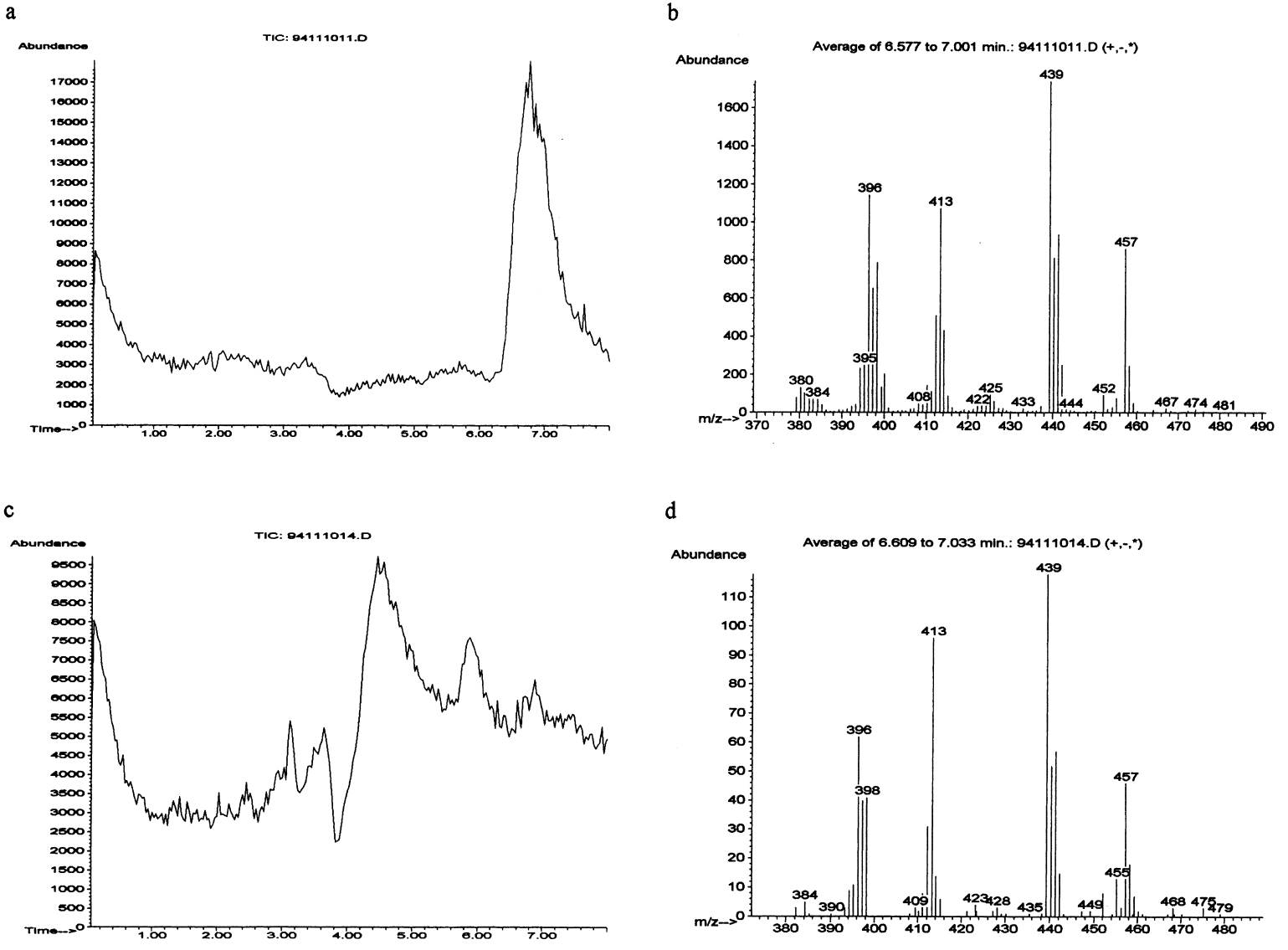


Fig. 6. Scan confirmation of incurred Mino. (a) TIC of standard; (b) NICI mass spectrum of Mino standard; (c) TIC of incurred Mino (32 ppb by determinative procedure analysis) milk extract; (d) spectrum of incurred Mino.

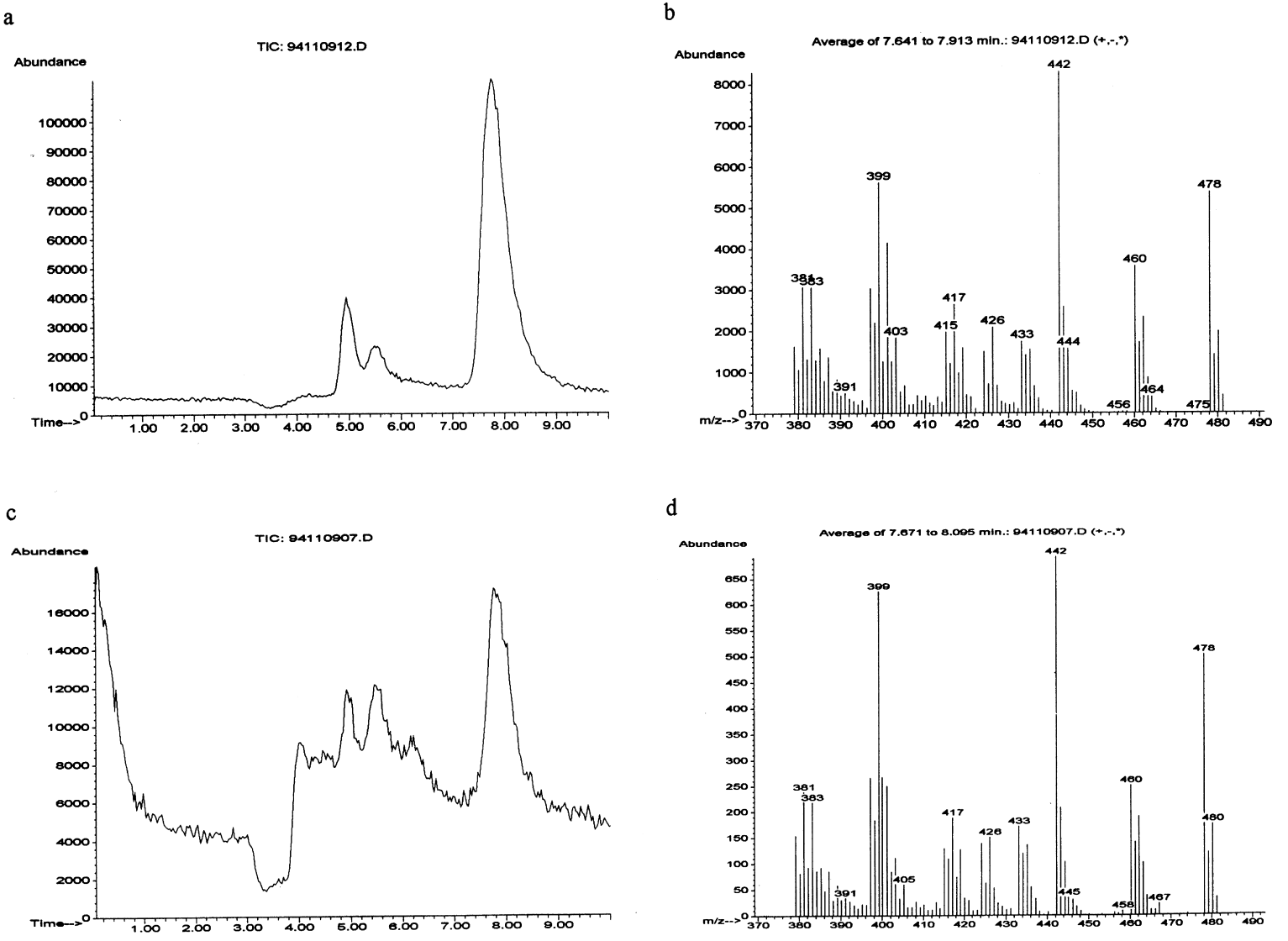


Fig. 7. Scan confirmation of incurred CTC. (a) TIC of standard; (b) NICI mass spectrum of CTC standard; (c) TIC of incurred CTC (31 ppb by determinative procedure analysis) milk extract; (d) spectrum of incurred CTC.

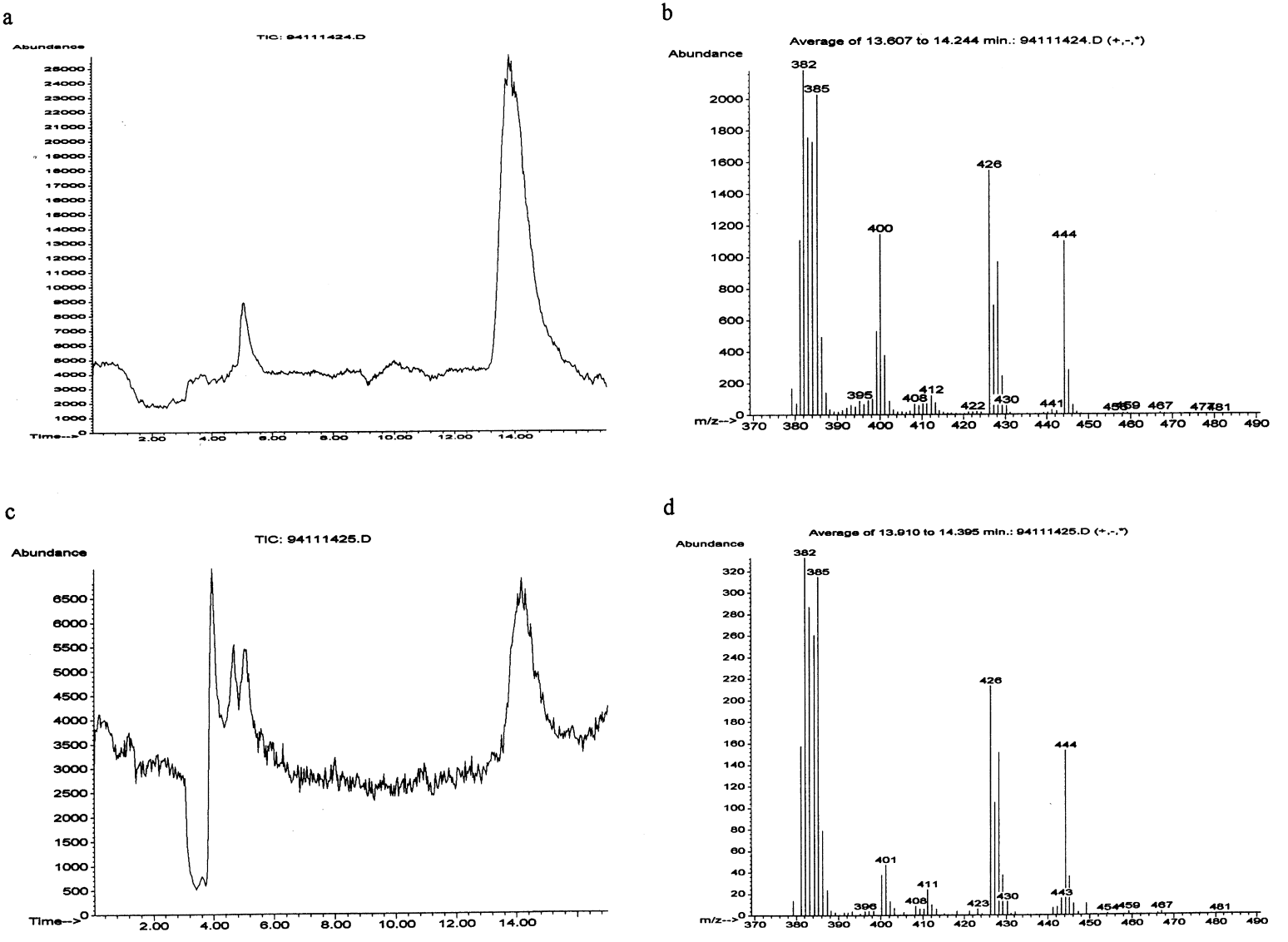


Fig. 8. Scan confirmation of incurred Doxy. (a) TIC of standard; (b) NICI mass spectrum of Doxy standard; (c) TIC of incurred Doxy (43 ppb by determinative procedure analysis) milk extract; (d) spectrum of incurred Doxy.

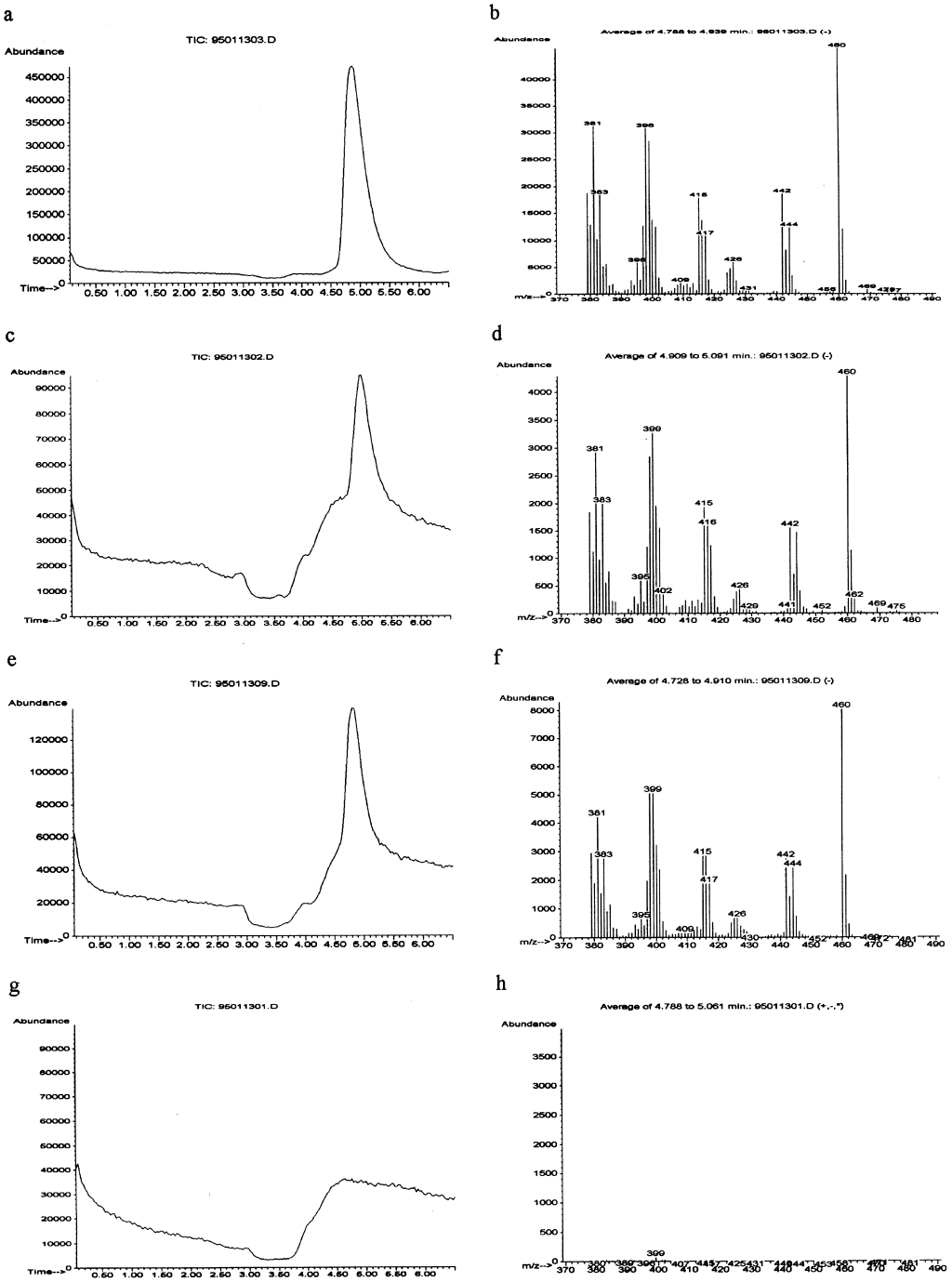


Fig. 9. Confirmation of fortified and incurred OTC residues in Ecuadoran white shrimp. (a) Total ion current (TIC) of standard injection; (b) NICI mass spectrum of OTC standard; (c) TIC of extract of 100 ppb OTC fortified white shrimp tissue; (d) spectrum of fortified OTC; (e) TIC of extract of white shrimp containing incurred OTC (118 ppb by determinative procedure analysis) residues; (f) Spectrum of incurred OTC; (g) TIC of extract of control white shrimp; (h) averaged and background-subtracted spectrum from control white shrimp extract at OTC retention time.

were all replicated a minimum of five times. All fortified and incurred milk samples confirmed only for the residue present, and all control milk failed to confirm.

Fig. 9 shows the confirmation of OTC residues in white shrimp. Confirmation of OTC fortified at 100 ppb is illustrated in Fig. 9, panels c and d. Naturally incurred OTC residues in the dosed white shrimp were also readily confirmed (panels e and f). Comparable results were observed for fortified black tiger shrimp. Neither control black tiger shrimp nor control white shrimp had any detectable interferences (Fig. 9, panels g and h).

The white shrimp had been ground with shell attached using the dry ice procedure [17]. Black tiger shrimp was ground using a conventional meat grinder after removing the shells. The ground shrimp tissue prepared using dry ice was easier to homogenize and produced a more even suspension than the shrimp tissue prepared with the meat grinder. However, the approximate recoveries of OTC from fortified ground shrimp were the same using the two grinding procedures. In both instances all fortified OTC residues were confirmed, and there were no interferences present in the control shrimp extracts. Samples of control and 100 ppb OTC fortified black tiger shrimp were analyzed three times. Samples of control, fortified, and dosed white shrimp were analyzed a minimum of five times.

4. Conclusions

MCAC and polymeric SPE provide the isolation and enrichment of TCC residues from diverse biological matrices which enable their confirmation at low ppb levels. LC–PB-MS with NICI using mass scanning mode can be used to confirm the residue identity of multiple members of the tetracycline class of drugs. TCCs have a stable core structure, making production of structurally diagnostic fragment ions difficult under commonly used MS conditions (ESI, APCI, even MS–MS). Particle beam NICI produces greater fragmentation and richer spectra, providing higher specificity of residue identification than has been previously reported for tetracycline analysis [10,11,22]. If greater sensitivity is required due to differences in instrumentation or regulatory need, confirmation by SIM is also a possibility, particularly if only one residue must be confirmed. However, in this case, care must be exercised in the selection of ions monitored to ensure that relative abundances of fragment ions in extracts match those from residue standards.

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