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Determination of Isopropyl Thioxanthone (ITX) in Fruit Juices by Pressurized Liquid Extraction and Liquid Chromatography−Mass Spectrometry

GIANNI SAGRATINI,[†] JORDI MAÑES,[§] DARIO GIARDINÁ,[†] AND YOLANDA PICÓ*[,§]

Dipartimento di Scienze Chimiche, Facoltà di Farmacia, Università degli Studi di Camerino, via S. Agostino 1, 62032 Camerino (MC), Italy, and Laboratori de Bromatologia i Toxicologia, Facultat de Farmàcia, Universitat de València, Avenida Vicent Andrés Estellés s/n, 46100 Burjassot, València, Spain

A rapid LC-MS method, which compares different mass analyzers—single quadrupole, ion trap, and triple quadrupole—was developed for the quantitative determination of isopropyl thioxanthone (ITX) in fruit juices. ITX, a photoinitiator in UV-cured inks that can reach foods from the cartons for beverages in which they are used, was extracted from fruit juice samples with acetone/hexane (50:50) using pressurized liquid extraction. This method gave detection limits of 3, 3, and 0.01 μg/L and quantification limits of 10, 10, and 0.05 μg/L using single quadrupole, ion trap, and triple quadrupole, respectively. Five replicate fortifications of different fruit juices at the quantification limit gave recoveries oscillating between 68 and 72% with CV varying between 14 and 18%. This is the first report of a positive mass spectrometric identification and quantification of ITX in fruit juice samples packed in TetraPack. The sensitivity and specificity of the LC-MS/MS analysis using the triple quadrupole enables it to be the method of choice for risk assessment and monitoring. The method was applied to apricot, orange, peach, apple, grape and pineapple, and cherry and strawberry juices and to fruit nectars from Spain and Italy, and the ITX was detected in the range of 0.05−0.78 μg/L.

KEYWORDS: Isopropyl thioxanthone (ITX); pressurized liquid extraction (PLE); liquid chromatography−mass spectrometry; electrospray; single quadrupole; ion trap; triple quadrupole; fruit juices

INTRODUCTION

In the past decade, food safety alarms have turned into a frequently recurring incident, which have led to public and political concern about the safety of food components. As a result of media attention, expressions such as "mad cow disease", "dioxin chickens", "MPA crisis", and "chloramphenicol scandal" are familiar to the general public. In the European Union (EU), consumer protection ranks extremely high according to the precaution principle (*1*) based on the Treaty of Amsterdam (*2*). Regularly, new alerts on possible food contaminants have to be considered. One of the most recently reported warning has been about isopropyl thioxanthone (ITX), a photoinitiator in UV-cured inks, triggering the radical polymerization of the acrylic components of such inks. This chemical can reach foods via various paths (*3*, *4*). During unrolling of the printed carton, it can migrate by means of spread (set-off) from the outside to the nonprinted inside of the package that comes into contact with the food. Furthermore, it can pass through packaging if no barrier layer, for instance, aluminum, has been applied. Notifications from the Italian authorities have shown the occurrence of the ink photointiator ITX in liquid milk products and cloudy fruit juices packaged in cartons. In studies into the levels of ITX, the European Food Safety Authority (EFSA) observed levels of ITX ranging from 27 to 440 μg/L in milk products and from <5 to 249 μg/L in fruit juices, fruit nectars, and drinks (*5*). Besides the EFSA, a German research institute also published positive findings of ITX in TetraPack fruit juices of 405 μg/kg (*6*).

Inks applied to food packaging materials are not covered by specific European legislation. However, materials and articles intended to come in contact with food should comply with the general criteria laid down in Article 3 of Regulation (EC) 1935/2004 (*7*); that is, they should not transfer their constituents to food in quantities that could endanger human health or bring about unacceptable changes in the composition of foodstuffs. These criteria are also reiterated in the Council of Europe Resolution AP (2005) 2 adopted in September 2005 on printed materials and articles intended to come in contact with food (*8*).

ITX has been tested with contradictory results in limited genotoxicity studies in vitro *(9)*; however, clearly negative results were obtained in two in vivo studies (*10*, *11*). As a consequence, the mutagenic in vivo effects in animal experiments cannot be confirmed, and no other toxicity data on ITX exist. Even though the toxicity evidence is not fully convincing, there is a need to have reliable data available to reach the

* Corresponding author (telephone +34 96 3543092; fax +34 96 3544954; e-mail Yolanda.Pico@uv.es).
[†] Università degli Studi di Camerino.
[§] Universitat de València.

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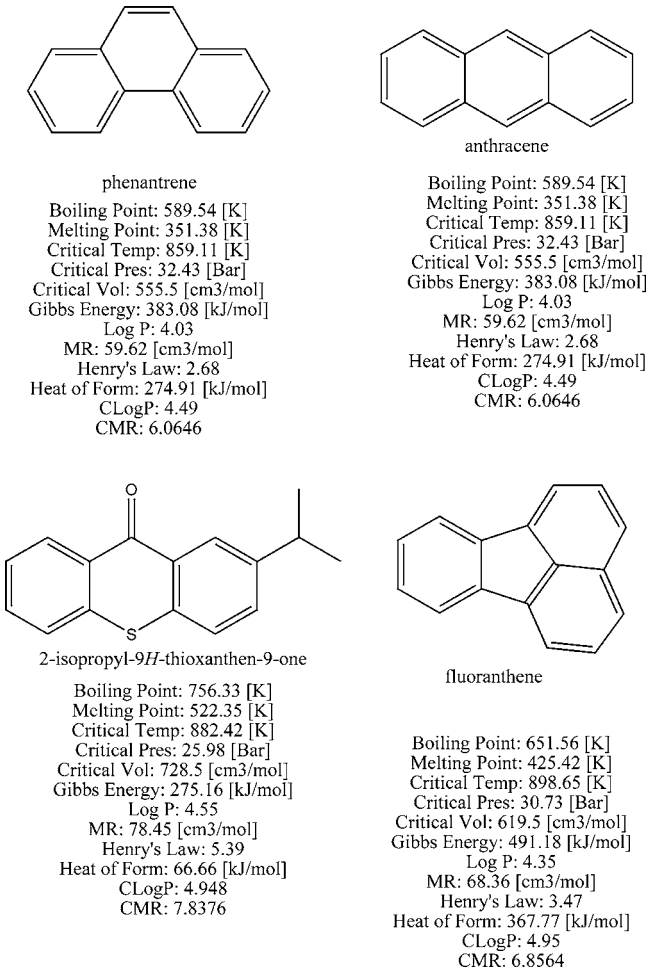

Figure 1. Structures and some chemical characteristics of isopropyl thioxanthone (ITX) and some typical polycyclic aromatic hydrocarbons (PAHs).

required level of protection through adequate risk evaluation and subsequent actions (*5*). This situation created a need for sophisticated, selective, sensitive, and robust analytical methods that can be used for the detection of ITX at low concentration levels in food. Several methods for the analysis of ITX have been developed (*12*, *13*). However, most analytical methods such as HPLC in combination with fluorescence detection or GC with mass spectrometry detection (MS) are not sufficiently sensitive (method detection limits of 5 and 2 μg/L, respectively, in milk-based food products) or specific to detect and quantify ITX in foods (*12*, *13*). The current trend related to the analytical determination of organic contaminants in food is not only to decrease the detection limits but also to increase the confirmatory character of the analysis in order to provide more confident regulation enforcement (*14*). As can be observed in the literature, the way to satisfy such a demand is the use of MS systems (*15*, *16*). Detection by electrospray ionization (ESI)-MS in combination with LC has not been described yet to determine traces of ITX in food.

Extraction of ITX has always been carried out by traditional organic solvent extraction (*12*, *13*). Solvents and procedural schemes have been selected assuming the high structural affinity between ITX and some polycyclic aromatic hydrocarbons (PAHs) such as phenantrene, anthracene, and fluoranthene (see **Figure 1**) (*13*). In fact, their lipophilicity expressed by log *P* is very comparable to that of isopropyl thioxanthone: log *P* ITX is 4.55, log *P* phenantrene is 4.03, log *P* anthracene is 4.03, and log *P* fluoranthene is 4.35. However, reported methods require large volumes of solvent and are time-consuming processes (*17*). For the analysis of PAHs alternative techniques have been developed and applied in the past 10 years, such as supercritical fluid extraction (SFE) (*18*), microwave-assisted extraction (MAE) (*19*), and pressurized liquid extraction (PLE) (*20*−*22*). Among these techniques, PLE has been confirmed as the most effective for PAH extraction from food samples (*23*, *24*). Up to now, the application of PLE to ITX has been reported only once to determine ITX in milk, yogurt, and fat by high-performance thin layer chromatography−mass spectrometry (HPTLC-MS) (*25*).

The aim of this study was to develop a method for the quantification and confirmation of trace amounts of ITX in fruit juices, based on the use of PLE, previous to LC-ESI-MS. Three different mass analyzers, single quadrupole, ion trap, and triple quadrupole, were compared. The method was applied to fruit juices taken from the Camerino (Italy) and Valencia (Spain) local markets.

MATERIALS AND METHODS

Materials and Standards. Isopropyl-9*H*-thioxanthen-9-one, $C_{16}H_{14}OS$, molecular mass 254 Da, 97%, mixture of 2- and 4-isomers, CAS No. 5495-84-1 and 84846-86-0, respectively, was supplied by Sigma-Aldrich (Madrid, Spain). Stock solution (1 mg/mL) was prepared by dissolving 100 mg of ITX into 100 mL of methanol. This solution was stored in stained, glass-stoppered bottles at 4 °C and was stable during the period of the study. Subsequent dilutions of the standard were daily prepared in methanol from the stock solution. Five-point calibration curves in apricot, orange, peach, apple, and fruit nectar extracts were prepared at LOQ, 2 × LOQ, 10 × LOQ, 20 × LOQ, and 100 × LOQ by adding aliquots of ITX standard to dry, negative control extracts, prepared as described below.

HPLC-grade methanol was supplied by Merck (Darmstadt, Germany), *n*-hexane from Scharlau (Barcelona, Spain), and acetone from VWR International (Madrid, Spain). Deionized water (<8 MΩ cm resistivity) was obtained from the Milli-Q SP reagent water system (Millipore, Bedford, MA). HPLC running solvents, standard solutions, and fruit juice extracts were filtered through 0.45 μm cellulose filters from Scharlau.

Sample Collection and Preparation. Thirty different fruit juice samples packaged in Tetra Brik Aseptic TetraPack (200 mL pack size) were taken from different supermarkets from the Camerino (Italy) and Valencia communities (Spain). Fruit juice samples were stored at room temperature and after their opening were stored into the specific food containers at 4 °C and analyzed at maximum in 3 days. Fresh fruit juice (1 mL) was blended with 2 g of sea sand (Panreac, Barcelona, Spain) and 7 g of Na_2SO_4 (Scharlau) for 5 min in a mortar using a pestle. This mixture was introduced into a stainless steel extraction cell (11 mL capacity), which was positioned in the PLE system connected to a four-bottle solvent controller, both from Dionex (Sunnyvale, CA). Nitrogen at a pressure of 10 bar was supplied to assist the pneumatic system and to purge the extraction cells. For the extraction, a mixture (50:50) of *n*-hexane and acetone (50% flush volume) was used at 100 °C and 10.4 MPa for 5 min of static time, in one cycle, preheated for 2 min, and purged for 60 s. Each PLE extract was concentrated to ∼1 mL in a Büchi R200 (Labortechnik, Flawil, Switzerland) rotary evaporator set at 40 °C and 0.02 MPa in a 50 mL round-bottom flask. Then, the extract was reconstituted in 2 mL of acetone.

LC-MS Analysis. The separation was achieved on a Luna C_{18} (150 × 4.6 mm i.d., 5 μm) analytical column preceded by a security guard cartridge C_{18} (4 × 2 mm i.d.), both from Phenomenex (Chesire, U.K.). The mobile phase was methanol/water at a flow rate of 0.3 mL/min. The solvent composition was 90% methanol and 10% water in isocratic mode. Liquid chromatography was performed using a Hewlett-Packard (Palo Alto, CA) HP-1100 series LC/MSD system consisting of an autosampler, a binary solvent pump, and an MSD, equipped with an ESI interface in positive ionization (PI) mode. Optimization of the LC-MS conditions was carried out by varying them in flow injection

analysis (FIA) of the ITX (20 μL of a 10 μg/mL standard solution). The optimized parameters of the interface were as follows: vaporizer temperature, 325 °C; nebulizer gas (nitrogen) pressure, 0.17 MPa; drying gas (nitrogen) flow rate, 13 mL/min, and temperature, 300 °C; capillary voltage, 4000 V; fragmentor, 90 V; gain, 1. Full-scan chromatograms were obtained by scanning from *m*/*z* 50 to 350, with a scan time of 0.75 s. Selected ion monitoring (SIM) was carried out for the most abundant ion of ITX (*m*/*z* 277) using high-resolution settings and a dwell time of 400 ms.

The LC-QIT/MS system consisted of an Esquire3000 Ion Trap LC/MS(*n*) system (Bruker Daltonik GmbH, Bremen, Germany), the Agilent HP1100 LC system, a computer, and a data acquisition/processing Daltonic Esquire Control Software system. The Esquire3000 was equipped with an ESI source that operated in positive ionization mode. The mass spectrometer was tuned for ITX compound, optimizing the ionization source parameters, voltages on the lenses, and trap conditions in the ExpertTune mode of the Daltonic Esquire Control software while infusing a standard solution (10 μg/mL) via a syringe pump at a flow rate of 4 μL/min, which was mixed with the mobile phase at 1 mL/min by means of a T piece. Operating conditions of the source were the same as in the single quadrupole. The lens and block voltage selected were as follows: skimmer, 4.0 V; capillary exit, 113.5 V; octopoles 1 and 2, 12 and 1.7 V, respectively; trap driver, 26.5%; octopole reference, 120%; and lenses 1 and 2, −5 and −60 V, respectively.

The mass spectrometer was run in full-scan and MRM modes. Positive ions were detected using the standard scan at normal resolution [scan speed = 10300 (*m*/*z*)/s; peak width = 0.6 fwhm/(*m*/*z*)]. The trap parameters were set in ion charge control (ICC) using rolling averaging set at 2 with a target of 20000 and maximum accumulation time of 50 ms at *m*/*z* range from 50 to 350 u. Collision-induced dissociation (CID) was performed on the ion of interest by collisions with the helium background gas present in the trap for 40 ms. The fragmentation steps for ITX were optimized by visualizing the changes in the intensities of fragment ions, whereas the fragmentation cutoff and the fragmentation amplitude were manually varied. The first fragmentation step (MS/MS) was carried out by isolating the ion at *m*/*z* 255 with a width of 4.0 *m*/*z*, a cutoff of 80 *m*/*z*, and an amplitude of 1.0 V and the second one (MS^3) by isolating the ion at *m*/*z* 213 with a width of 4.0 *m*/*z*, a cutoff of 80 *m*/*z*, and an amplitude of 1.5 V.

A TQ mass spectrometer Quattro LC from Micromass (Manchester, U.K.), equipped with an LC Alliance 2690 system (Waters, Milford, MA) consisted of an autosampler and a quaternary pump, a pneumatically assisted electrospray probe, a Z-spray interface, and Mass Lynx NT software ver. 4.1 used for the MS/MS analyses coupled to the Waters LC 2695 separation module. Parameters were optimized by continuous infusion of a standard solution (20 μg/mL) via a syringe pump at a flow rate of 15 μL/min. Analysis was performed in both positive and negative ion modes (the positive or negative polarity of some voltages change according to the ionization mode). The ESI source values were as follows: capillary voltage, 3.20 kV; extractor, 5 V; RF lens, 0 V; source temperature, 100 °C; desolvation temperature, 300 °C; desolvation gas (nitrogen, 99.99% purity) flow, 500 L/h. The analyzer settings were as follows: resolution, 12.0 (unit resolution) for the first and third quadrupoles; ion energy, 0.9; entrance and exit energies, 1 and 2; multiplier, 650; collision gas (argon, 99.995%) pressure, 2.79×10^{-7} MPa; interchannel delay, 0.02 s; total scan time, 1.0 s. The mass spectrometer was operated in scan, product ion scan, and multiple reaction monitoring (MRM) modes. In MRM the transitions of *m*/*z* 255 to *m*/*z* 213 and *m*/*z* 255 to *m*/*z* 184 were monitored at a cone voltage (CV) of 40 and a collision energy (CE) of 25 with a dwell time of 0.5 s for both transitions.

Method Validation. The method was validated by analyzing control fruit juices fortified with ITX at concentrations of 0.01, 0.05, 0.1, 0.5, 1.0, 3.0, 5.0, 10, 20, 100, 200, and 1000 μg/L (detailed concentrations used in each instrument are shown in **Table 1**). The fortifications were prepared by adding 50 μL of different ITX standard solutions in methanol (concentrations between 0.2 μg/mL and 20 mg/mL) to 10 mL of fruit juice. These samples were mantained for 15 min under continuous agitation, and then they were allowed to stand at room temperature for 15 min prior to extraction. The method was carried out in triplicate to evaluate the selected quality parameters, which were linearity, recoveries, repeatability, and sensitivity.

Table 1. Validation Results of ITX Determination in Apricot Juices through the Whole Procedure Using the Different LC-MS Instruments

			within day			between days		
instrument	LOD (μg/L)	fortification level (μg/L)	recovery (%)	RSD (%)	*N*	recovery (%)	RSD (%)	*N*
single quadrupole	3	10[a]	74	15	5	70	17	5
		20	76	14	5	73	16	5
		100	77	12	5	77	15	5
		200	75	8	5	79	12	5
		1000	80	7	5	83	12	5
QIT	3	10[a]	72	16	5	72	18	5
		20	76	16	5	72	16	5
		100	75	11	5	74	14	5
		200	78	10	5	78	10	5
		1000	77	8	5	76	11	5
TQ	0.01	0.05[a]	69	14	5	68	16	5
		0.1	72	14	5	74	17	5
		0.5	74	11	5	73	14	5
		1	78	12	5	77	15	5
		5	77	10	5	77	13	5

[a] Fortification level corresponding to the LOQ.

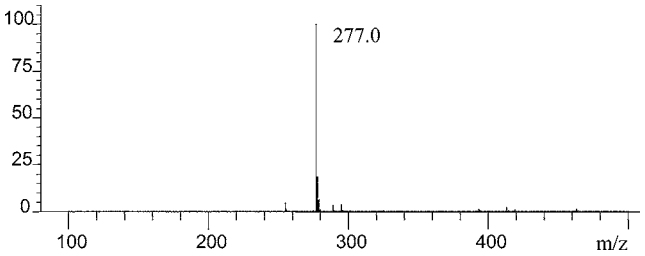

Figure 2. ESI full-scan mass spectrum in positive ionization mode of ITX standard (200 ng injected) using single quadrupole.

RESULTS

Evaluation of PLE Conditions. PLE conditions were tested experimentally to assess the influence of the exposure time (1, 5, or 10 min), the PLE temperature (40−200 °C), and the ratio of hexane/acetone (30:70, 50:50, and 70:30) on the ITX extraction yields. Preliminary experiments showed that high recoveries could be achieved with only one PLE cycle. Extraction efficiency decreases, changing the ratio of *n*-hexane/acetone (70:30), reaching recovery values of 30%. When the temperature rose to 120 °C, the recovery diminished to 54%, in the same way using *n*-hexane/acetone (50:50) with 80% flush volume. After 5 min of static time, the recovery was the maximum achievable. Sodium sulfate and diatomaceous earth were tested as drying agents. The latter attained lower efficiency in the extraction, expressed by a recovery of 49%. ITX was extracted from fruit juices using a mixture of *n*-hexane/acetone (50:50) with 50% flush volume at 100 °C and 1500 psi for 5 min static time in one cycle similar to the conditions reported for some PAHs (*23*, *24*). These conditions provide an average recovery of 73%.

Single-Quadrupole, Quadrupole Ion Trap, and Triple-Quadrupole Mass Spectra of ITX. Figure 2 illustrates the full-scan ESI^+ mass spectrum of ITX obtained with the single quadrupole. The most abundant fragment was the sodium adduct $[M + Na]^+$ at *m*/*z* 277. The sodium adduct is typical of molecules that have oxygen or hydroxyl atoms that have a pair of electrons and is caused by traces of Na^+ present in the HPLC

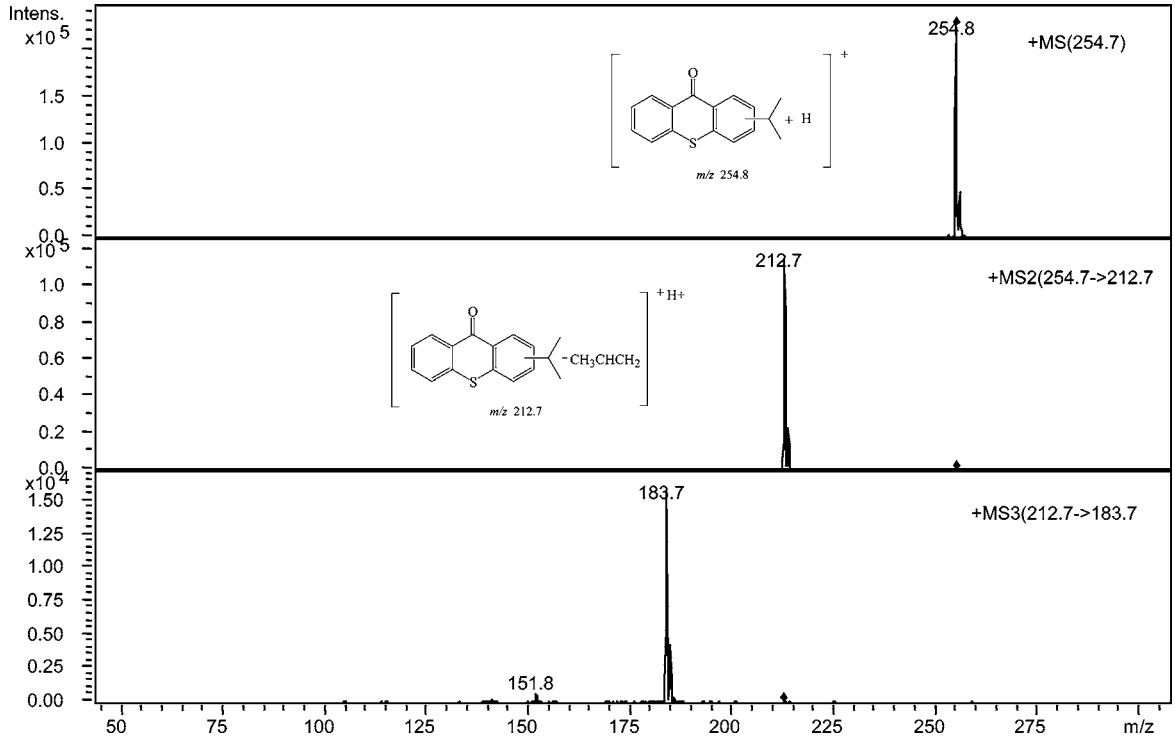

Figure 3. Precursor and product (MS^2 and MS^3) ion scan mass spectra of ITX standard obtained in the ion trap (amount injected = 200 ng).

system (solvents, tubes, etc.). Using this instrument, the sodium adduct was always the most important, even after trying different types of Milli-Q waters or various methanol brands.

Figure 3 shows the full-scan ESI^+ ITX mass spectrum and product ion scan mass spectra (MS^2 and MS^3) obtained by quadrupole ion trap. As a contrast with the single quadrupole, the only precursor ion observed operating in full scan was the protonated molecule $[M + H]^+$ at *m*/*z* 254.8. The MS^2 spectrum of $[M + H]^+$ evidences an intense signal at *m*/*z* 212.7 that corresponds, according to the proposed fragmentation, with the ion produced by neutral loss of propene (*m*/*z* 42.1). The fragmentation of this ion produced an MS^3 spectrum characterized by a not very intense signal at *m*/*z* 183.7, which can be a result of a strange ionization (EI type).

Figure 4 reports the triple-quadrupole mass spectra corresponding, respectively, to the MS scan and the product ion scan of ITX. The MS scan spectrum shows clearly as the highest signal is referred to $[M + H]^+$ at *m*/*z* 255.0, similar to the ion trap mass spectrum. The difference with QIT is the coexistence of the sodium adduct $[M + Na]^+$ at *m*/*z* 277 with the protonated molecule. The MS/MS transition of selected precursor ion produces a mass spectrum characterized by the most abundant fragment at *m*/*z* 213.0 and a minor fragment at *m*/*z* 184 as in the QIT. Under the developed methodology, the instrumental detection limits, calculated on the basis of noise level (S/N = 3) and proved by injecting standard solutions at those concentrations, were 1.5 ng/μL for single quadrupole and ion trap using MS/MS and 0.005 ng/μL for the triple quadrupole.

Comparison of Method Validation. The performance of the method was evaluated using three different detectors: single quadrupole, ion trap, and triple quadrupole. The results are presented in **Table 1** for appricot juices. Results obtained for other fruit juices do not reveal any appreciable difference (data not shown). The data demonstrate that ITX in triple quadrupole exhibits a very low LOD of 0.01 μg/L and consequently a very significant LOQ of 0.05 μg/L. In contrast, the single quadrupole and the ion trap gave the LOD and LOQ higher than TQ, the same for both and, respectively, 3 and 10 μg/L. The good recoveries obtained are similar for three different instruments and ranged from 68 to 83% with RSDs of 7−18%. The linearity was tested in apricot, orange, peach, apple, and fruit nectar extracts at LOQ, 2 × LOQ, 10 × LOQ, 20 × LOQ, 100 × LOQ, and optimum values of R^2 were obtained by calculating it in three different instruments: 0.999 for single quadrupole, 0.996 for QIT, and 0.997 for TQ.

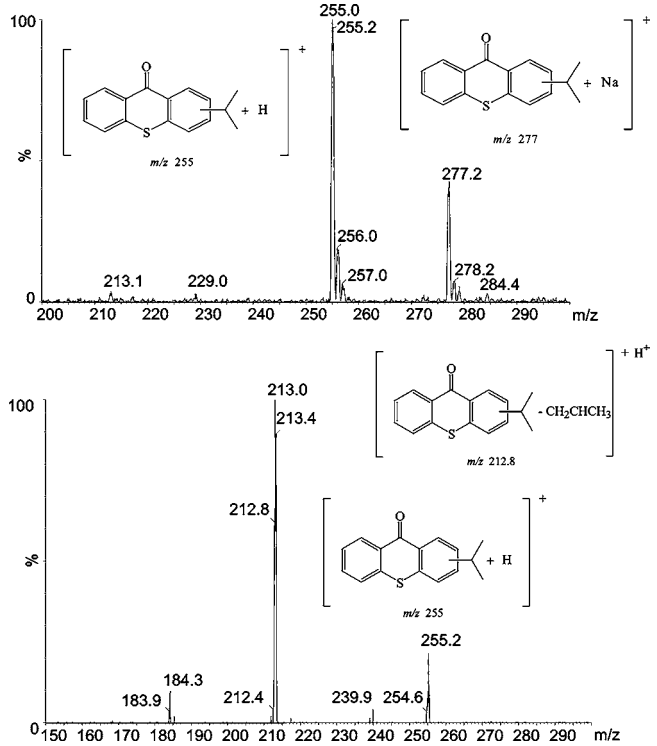

Figure 4. Precursor and product ion scan mass spectra of ITX standard obtained in the triple quadrupole (amount injected = 200 ng).

It was also evaluated how the matrix effect can affect the final results; the matrix effect was evaluated by comparison of

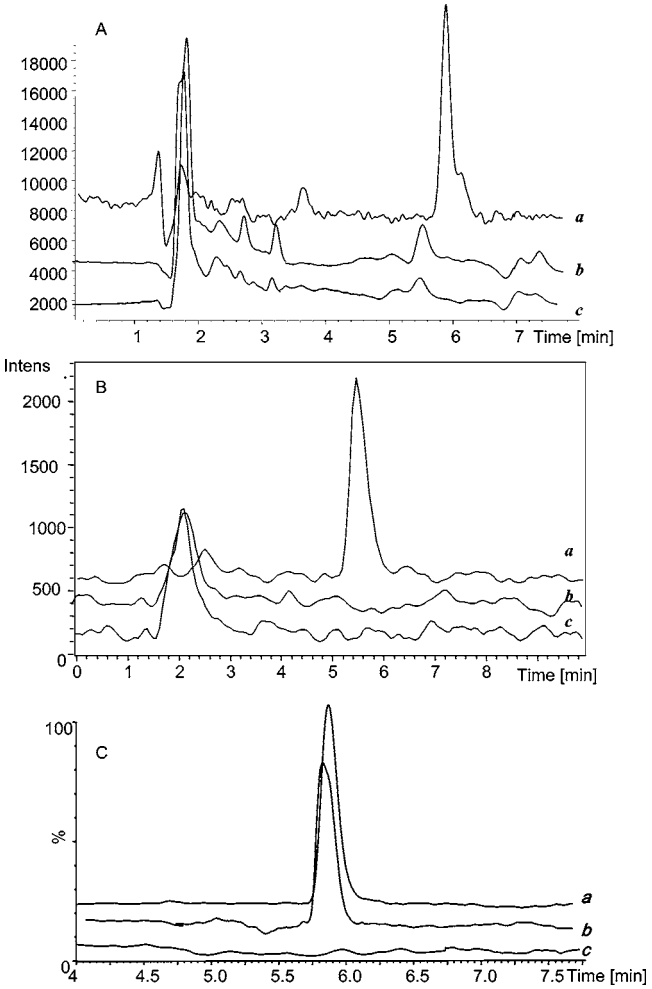

Figure 5. Representative determinative LC-MS chromatograms obtained by (**A**) single quadrupole, (**B**) ion trap, and (**C**) triple quadrupole of (a) 50 μg/L of ITX standard for single and ion trap quadrupoles and 1 μg/L for triple quadrupole, (b) positive sample of apricot juice at 0.78 μg/L of ITX, and (c) negative sample of apricot juice

the response of ITX standard prepared in fruit juice extracts with standards in methanol, at LOQ and 10 × LOQ concentration. The isopropyl thioxanthone showed in the matrix obtained by PLE a low suppression in relation to the response obtained in pure solvent standard. This suppression was quantified in a value of 7%. Therefore, quantitation was performed using standards in matrices that matched those of the samples.

The limited sensitivity of LC-MS to analyze ITX in fruit juices using single quadrupole and ion trap MS/MS as well as the appropriate sensitivity of triple quadrupole to analyze real-contaminated samples is exemplified in **Figure 5**, where are reported three chromatograms for each instrument corresponding to (a) 50 μg/L of ITX standard in methanol for single quadrupole and ion trap and 1 μg/L for the triple quadrupole, (b) positive apricot juice at 0.78 μg/L of ITX, and (c) negative sample of apricot juice. The overlapping of three chromatograms shows that, at the retention time of ITX (6.2 min) in chromatogram b (positive sample), it is not possible identify the very low quantity of ITX using single quadrupole and quadrupole ion trap; in fact, the profile of chromatogram b is the same as that of the negative sample (c), and in both cases a weak signal at 5.9 min is presented, probably due to a matrix component, that could mislead. On the contrary, using the triple-quadrupole instrument, the peak corresponding to ITX is clearly observed without interfering peaks.

Table 2. ITX Detected in Italian and Spanish Fruit Juices

sample/ origin	juice fruit	fruit (%)	type of independent lot	result	quantity detected (μg/L)
1-I	apricot	40[a]	A	+	0.78
2-I	apricot	40	B	+	0.30
3-I	apricot	40	B	+	0.14
4-I	apricot	40	B	–	
5-I	apricot	40[a]	C	+	0.15
6-I	apricot	40[a]	C	–	
7-I	apricot	40[a]	C	+	0.09
8-I	apricot	35[a]	D	–	
9-I	apricot	35[a]	D	–	
10-I	apricot	50	E	–	
11-I	apricot	50	E	–	
12-I	apricot	50	E	–	
13-I	apricot	40[a]	F	–	
14-I	apricot	40[a]	F	–	
15-I	apricot	40[a]	F	–	
16-S	orange	50[a]	G	–	
17-S	peach	45	H	+	0.15
18-S	peach	45	H	–	
19-S	peach	45	H	-	
20-S	grape and pineapple	not reported	I	–	
21-S	grape and pineapple	not reported	I	+	0.30
22-S	grape and pineapple	not reported	I	–	
23-S	fruit nectars	50	J	+	0.05
24-S	fruit nectars	50	J	+	0.05
25-S	fruit nectars	50	J	+	0.09
26-S	apple	not reported	K	+	0.06
27-S	apple	not reported	K	–	
28-S	apple	not reported	K	–	
29-S	cherry and strawberry	50	L	–	
30-S	orange	not reported	M	–	

[a] Mininum quantity.

Another advantage of the triple quadrupole is that confirmation (qualitative identification) was based on the ion ratios of the MS/MS product ions in samples matching those in the standard spikes. The triple quadrupole provided consistent MS/MS ion ratios, allowing for a high level of confidence in ITX identification. The average peak ratio for *m/z* 184/213 was 0.15 (range = 0.12−0.18, CV = 5%) from analyses of 10 standards at a concentration of 0.0 5 μg/mL. This was compared to ion ratios for analyses of standards of the same concentration prepared in juice extracts (n = 10), where the average peak height ratio for *m/z* 184/213 was 0.13 (range = 0.10−0.17, CV = 8%). No significant differences in ion ratios caused by the juice matrix were observed. These data indicate that triple quadrupole can provide qualitative data that meet strict method performance criteria specified in the European Union guidelines for the analysis of toxic contaminants in animal products, which allow CV for relative intensitites of 20% (*26*). The other mass analyzers do not meet these criteria because they do not provide enough fragment ions.

The procedure was applied to the analysis of 30 fruit juices samples packed in TetraBrickAseptic TetraPack, 15 from Italy and 15 from Spain. In **Table 2** are reported the number of samples, the type of fruit juices, the fruit percentage, the independent lots (various brands), and the quantity of ITX detected expressed in micrograms per liter. In Italian fruit juices, 5 samples of a total of 15 analyzed contained ITX, with concentration values from 0.09 to 0.78 μg/L. In Spanish juices, 6 samples of a total of 15 analyzed contained ITX, with levels that ranged from 0.05 μg/L (LOQ) to 0.30 μg/L. The contaminated samples were completely independent from the lot type (brand); in fact, in the case of the typical packet 3 × 200 mL

of fruit juices, not all of the units of the lot were contaminated (see cases of B and C lots in Italian juices and H, I, and K lots in Spanish juices).

There is a plausible explanation for the ITX contamination mechanism in some foods packed in TetraPack. It seems that the contamination happens during the rolling of the carton, when the external printed part comes in contact with the internal nonprinted part of the carton, remaining rolled and compressed in this way also for a month before the industrial packaging for food products. In a fatty food such as milk, the ITX non polymer bonded is easily brought in solution because of the high lipophilicity of the molecule, whereas in the case of fruit juices, which normally contain a low percentage of fats, the fibers present in juice could incorporate the photoinitiator and so contaminate the sample.

The combination of LC with different mass spectrometers—single quadrupole, ion trap, or triple quadrupole—provided, in all cases, reliable quantification. The sensitivity and specificity of the triple-quadrupole mass spectrometer make the described method a powerful tool for toxicological and public health investigation in food safety. This method is a significant improvement over the existing methodology in that it provides a rapid and unequivocal determination of ITX in fruit juices, and it is validated at very low part per billion concentrations. Additionally, it offers the first report of a positive LC-MS identification and quantification of ITX in fruit juice samples, along with firm quality control measures. Batches of 50 fruit juice samples can be easily analyzed by one analyst in a day.

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