Determination of sub-ppb epichlorohydrin levels in water by on-line solid phase extraction-liquid chromatography-tandem mass spectrometry

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ABSTRACT

A new sensitive and selective method based on on-line solid-phase extraction (SPE) coupled to LC(ESI)MS/MS using a triple quadrupole analyzer has been developed for the determination of epichlorohydrin (ECH) in different types of water samples. The great difficulties for ECH direct determination resulting from its low molecular size, high polarity and non-easily ionizable molecule make necessary a previous derivatization step. This previous reaction was optimized employing 3,5-difluorobenzylamine as derivative agent adding Fe(III) to catalyze the derivatization process. In order to achieve accurate quantification and for correction of matrix effects, losses in the derivatization process and instrumental deviations, ECH isotope labelled (ECH-d_5) was added as internal standard (IS) to water samples. The method was validated based on European SANCO guidelines using drinking and other types of treated water spiked at two concentration levels (0.1 and 1.0 µg/L), the lowest having been established as the limit of quantification (LOQ) objective of the method. Satisfactory accuracy (recoveries between 70 and 103 %), precision (RSD < 20 %) and linearity (from 0.05 to 50 µg/L, r > 0.99) were obtained. The limit of detection (LOD) was set-up at 0.03 µg/L. The method was applied to different water samples (drinking water and water samples collected from a municipal treatment water plant). In order to enhance confidence, five SRM transitions were acquired obtaining in this way a simultaneous reliable quantification and identification of ECH in water, even at sub-ppb levels.

Keywords

Epichlorohydrin, liquid chromatography, tandem mass spectrometry, water, confirmation.

INTRODUCTION

Epichlorohydrin (1-chloro-2,3epoxy-propane, ECH) is an aliphatic epoxide commonly employed as starting material in the production of synthetic glycerol, plastics, polymers and epoxy resins. ECH residues can enter in drinking-water supplies through different ways, as it is widely employed in the fabrication of drinking-water pipes as well as in the synthesis of cationic polyelectrolytes, which are used in surface and wastewater clarification, and in several flocculating agents[1].

ECH is toxic by inhalation, dermal and oral absorption, and it is defined as probably carcinogenic to humans (group 2A) by the International Agency for Research on Cancer (IARC)[2]. Due to its toxicity, ECH has been listed among compounds dangerous to the water environment by both EU and USA[3,4]. According to European Council Directive 98/83/EC on the quality of waters intended for human consumption, the acceptable limit for ECH in drinking water is 0.1 μg/L[3]. Stricter is the maximum level contaminant (MLC) goal established by US Environmental Protection Agency, which has been set at zero[4]. Therefore, it is necessary the development of highly sensitive analytical methodology able to determine ECH at sub-ppb levels in water.

Nowadays, no practical routine and confident analytical methods are available to determine ECH at such low concentrations. Chemical characteristics of ECH, like high solubility in water, volatility and polar character make very difficult its analysis. Furthermore, the hydrolytic behavior of this substance has to be taken into account since its presents a half life in water at pH 7 and 20 ºC of only 6.2 days[5], which is lower at other pH values. Moreover, ECH hydrolysis increases 7-fold when the temperature exceeds 40 ºC[1].

ECH similarly to other volatile organic compounds has been determined in water by gas chromatography (GC), which requires multi-stage and time-consuming procedures previous to the chromatographic analysis. Methods described are most often based on isolation and/or enrichment techniques as dynamic headspace purge and trap[6,7], static headspace[7,8], LLE[8], SPE[5,8], or SPME[8-10].

GC determination has been carried out by using detection systems as ECD[5,7,9,12], FID[9,10,12] and MS[10-13].

In general, the sensitivity of the reported methods is insufficient for regulatory purposes and in most of cases, the reliable identification of ECH is not ensured (e.g. when using ECD, FID). Lucentini *et al.*[7] reported a purge and trap method for drinking water, which was validated at 0.1 µg/L, although the detection was based on GC/ECD.

Gaca and Wejnerowska[12] compared different GC methods for ECH determination in water. Direct aqueous injection and different extraction methods (headspace, striping with adsorption on solid phase, LLE, SPE and SPME) and detectors (FID, ECD, MS) were compared regarding sensitivity, using aqueous standards. They concluded that SPME followed by GC/ECD led to the lowest LODs. The calibration was plotted at the range of concentrations from 4.8 to 400 µg/L.

Khan *et al.*[13] have performed a detailed study of the potential of aqueous-phase aminolysis for the determination of epoxides, considering also the identification performance when using GC with quadrupole mass selective detector. A method was proposed for the determination of ECH in water based on a previous aminolysis reaction with 3,5-difluorobenzylamine (DFBA), solid phase extraction of the DFBA-derivatized samples, followed by silylation of the extract before GC/MS analysis in mode selected ion monitoring (SIM). This was a laborious procedure that required the use of a surrogate standard in order to obtain a reliable method. For this purpose, a chemical analogous compound as epifluorhydrin was selected allowing to reach a LOD of 10 ng/L.

Recently, ECH has been determined by GC-MS in food contact surface of epoxy-coated cans by Sung *et al*[14], after previous extraction with dioxane and derivatization with cyclopentanone and borontrifluorodiethyletherate.

Considering the high solubility in water and polar character of ECH, it seems more advisable the use of liquid chromatography (LC) instead of GC for its determination in water. Thus, Sarzanini *et al.*[15] performed a derivatization reaction with sulfur (IV) (added as anhydrous sodium sulfite) to obtain a product with a terminal sulfonate group, which was suitable to be retained in

suppressed anion-exchange chromatography. Despite the previous SPE pre-concentration step using polystyrene-divinylbenzene cartridges, the use of a low selective and sensitive detection technique such as conductivity, did not allow to reach a satisfactory sensitivity, and detection limit was established at 0.6 µg/L. Later, the method selectivity was improved by applying the same reaction, but pre-concentrating with C_{18} SPE cartridges and using ion chromatography with MS detection[16]. Five different reaction products were identified, and the LOD was estimated to be 2 µg/L for the most stable specie, due to the presence of interferences.

Tandem mass spectrometry (MS/MS) coupled to LC has became the most appropriate and sensitive technique to analyse many medium-high polar organic pollutants in water, leading to satisfactory results from both quantification and confirmation purposes[17,18]. The high sensitivity and selectivity of LC/MS/MS can even allow direct injection of water samples, reaching low LODs for many compounds[19,20]. However, a pre-concentration step, normally by solid-phase extraction (SPE), is usually required for the satisfactory determination of sub-ppb levels in multi-residue analysis where a variety of water pollutants like pharmaceuticals[21-24], drugs[24-26] and pesticides[17,23,24,27] have to be determined. The SPE preconcentration can be easily performed in on-line mode facilitating automation in SPE/LC/MS/MS methods[17].

In spite of analytical advantages offered by LC/MS/MS, there are still several highly polar compounds, whose determination requires special effort. Thus, large volume injection together with a detailed ionization process optimization was required to quantify and confirm acrylamide residues in water at sub-ppb levels[28]. In other cases, ion-pairing reagents have been required to favour retention in reverse-phase chromatography, thus allowing direct injection of sample and avoiding laborious sample treatments[29]. Other polar compounds, like glyphosate and gluphosinate, required a previous derivatization reaction for their determination in water[30].

The purpose of this paper was to develop a new selective and sensitive method based on on-line SPE/LC/MS/MS for ECH determination in water at sub-ppb levels, previous derivatization by an aqueous-phase aminolysis. The method was validated to ensure the accurate quantification and

identification of ECH at the low levels required by the EU drinking water legislation[3]. A special emphasis was made to obtain reliable and safe analyte identification by acquiring several selected reaction monitoring (SRM) transitions to reach an adequate number of identification points (IPs) [31].

EXPERIMENTAL

Reagents and Chemicals

ECH reference standard (99.5%) was purchased from Dr. Ehrenstorfer (Augsburg, Germany) through Scharlab (Barcelona, Spain) and ECH-d_5 (≥98%) was supplied by Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Terbuthylamine (99.5%) (tBA), 3,5-difluorobenzylamine (96%) (DFBA) and ferric chloride hexahydrate (99%) ($FeCl_3 \cdot 6H_2O$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetic acid (>99%) (HAc), formic acid (>98%) (HCOOH), ammonium acetate (98%) (NH_4Ac), acetone for residue analysis, HPLC-grade acetonitrile (ACN) and methanol (MeOH) were purchased from Scharlab. HPLC-grade water was obtained by purifying demineralised water in a Milli-Q Gradient A10 (Millipore, Bedford, MA, USA).

Stock standard solutions of ECH and ECH-d_5 were prepared by dissolving the pure compound in acetone obtaining a final concentration of 10000 mg/L. Intermediate standard solutions at concentration down to 10 mg/L were prepared from stock solutions by dilution with acetone and stored in a freezer at < -18 ºC. Working solutions were prepared daily at various concentrations by diluting with HPLC-grade water the intermediate standard solutions.

Instrumentation

A Quattro Micro triple quadrupole mass spectrometer (Waters, Milford, MA, USA) was interfaced using an orthogonal Z-spray-electrospray ion source to an HPLC system based on a Waters Alliance 2695 (Waters) quaternary pump used for the chromatographic separation, a 233XL autosampler with a loop of 2.5 mL (Gilson, Villiers-le-Bel, France) and a Varian 9012 (Varian, Palo Alto, USA) binary pump used to condition and wash the SPE cartridge.

Nitrogen generated from a pressurized air in a high-purity nitrogen generator (NM30LA 230Vac Gas Station from Peak Scientific, Inchinnan, UK) was employed as drying and nebulising gas. The cone gas and the desolvation gas flows were set to approximately 60 L/h and 600 L/h,

138 respectively. For operation in MS/MS mode, collision gas was Argon 99.995% (Praxair, Valencia,
139 Spain) with a pressure of approximately 3×10^{-3} mbar in the collision cell. Electrospray needle
140 capillary voltage of 3.5 kV was selected in positive ionization mode. The desolvation temperature
141 was set to 350 ºC and the source temperature to 120 ºC. Infusion experiments were performed using
142 the built-in syringe pump directly connected to the ion source at a flow rate of 10 µL/min. Dwell
143 times of 200 ms and scan ranges between 50 and 300 m/z were chosen. A solvent delay of 9 min
144 was selected to give an additional clean-up using the built-in divert valve controlled by the
145 Masslynx NT v 4.0 software (Waters).

146 Cartridges used for off-line SPE experiments were Oasis HLB (0.2 g) from Waters. For on-
147 line experiments, C_{18} and polymeric phase Hamilton (PRP), both 10 x 2 mm, 10 µm (Teknokroma,
148 Barcelona, Spain), and Oasis HLB 20 x 2.1 mm, 25 µm (Waters) cartridges were tested.

149 LC columns tested for chromatographic separation were: Discovery 50 x 2.1 mm, 5 µm
150 (Sigma); Sunfire 50 x 2.1 mm, 3.5 µm and 5 µm (Waters); Sunfire 100 x 2.1 mm, 3.5 µm (Waters);
151 Atlantis 50 x 2.1 mm and 100 x 2.1 mm, both 5 µm (Waters).

152 Masslynx NT v 4.0 (Waters) software was used to process the quantitative data obtained
153 from calibration standards and from water samples.

154

155 **Recommended procedure**

156 The derivatization procedure was performed by adding 20 µL of DFBA, 20 µL of
157 $FeCl_3\cdot6H_2O$ aqueous solution (6 g/L) and 80 µL of ECH-d_5 (500 µg/L) to 20 mL of water sample,
158 in amber glass vials, leaving them overnight at room temperature. Then, the derivatized samples
159 were filtered through 0.45 µm nylon filters before chromatographic analysis to remove undesirable
160 water particles and iron traces. A 2.5 mL aliquot of derivatized sample was directly injected into the
161 SPE/LC(ESI)MS/MS system using a C_{18} cartridge, 10 x 2 mm, 10 µm (Teknokroma) for
162 preconcentration, and a Sunfire C_{18} column, 50 x 2.1 mm i.d., 5µm particle size (Waters) for
163 chromatographic separation.

On-line SPE/LC was performed as follows: firstly, the SPE cartridge was conditioned with acetonitrile at a flow rate of 1 mL/min for 1 min, following by 1 min of water. An aliquot of 2.5 mL of derivatized sample was pre-concentrated into the cartridge and it washed with water at 1 mL/min for 3 min. Then, the sample was transferred in backflush mode to the analytical column, starting the LC gradient. A binary water / methanol (both 0.1 mM NH_4Ac) gradient elution was applied changing linearly the percentage of methanol as follows: 0 min, 5%; 2 min 5%; 5 min, 45%; 7 min, 90%; 8 min, 90%; 8.10 min, 5%. The flow rate was kept at 0.2 mL/min and the chromatographic run time was 15 min.

Quantification was performed by using the internal standard (IS) procedure, and calibration was carried out with standards prepared in water subjected to the same on-line preconcentration applied to the samples. ECH-d_5 was used as IS added to the water samples before the derivatization step. It was crucial to prepare all aqueous standard solutions daily due to the quickly degradation of this analyte in water.

Validation study

Method validation was performed following European SANCO guidelines recommendations[32]. Linearity was studied by injecting aqueous standards in triplicate at eight different concentrations, in the range from 0.05 to 50 µg/L. Satisfactory linearity was assumed when the correlation coefficient (r) was higher than 0.99, based on analyte peak areas measurement, and the residuals lower than 30 %.

Accuracy (expressed as recovery, in %) and precision (expressed as relative standard deviation, in %) were estimated by analyzing three types of water samples (drinking water treatment plant, DWTP; distribution system water, DSW; drinking water, DW) spiked at two concentration levels each: 0.1 µg/L and 1.0 µg/L. All recovery experiments were performed in triplicate for each type of water samples. Quantification was performed by internal calibration with standards in the range 0.05 – 2.5 µg/L for the low level and 0.05 - 10 µg/L for the high level. The

limit of quantification (LOQ) objective was established as the lowest concentration level that was validated with satisfactory results. The limit of detection (LOD) was estimated as the lowest concentration that the analytical procedure can reliably differentiate from background levels, and it was calculated for a signal-to-noise ratio of three from the chromatograms of samples spiked at the lowest analyte concentration tested.

The safe identification of ECH was carried out by quantification of the analyte using the quantification (*m/z* 236 > 92) and confirmation transitions (*m/z* 236 > 127, 236 > 218, 238 > 127, 238 > 94) and calculating the ratio between all calculated concentrations. Detection was considered as positive when these ratios fall in the range 0.8 to 1.2 (i.e. maximum concentration ratio deviation of ± 20%).

RESULTS AND DISCUSSION

In our first experiments, it was considered the use of two primary amines (tBA and DFBA) as aminolysis derivatizing agents for the determination of ECH in water samples. The epoxides ring opening is usually carried out by aminolysis at high temperatures or at room/low temperatures in the presence of a catalyst. Preliminary experiments indicated that tBA led to an unstable derivatization product, which was thermally degraded at room temperatures and even when the derivative was kept in the fridge. When DFBA was used as derivatizing agent, results were more satisfactory. In consequence, DFBA was selected and aqueous-phase aminolysis was carried out in presence of Fe^{3+} according to Khan *et al*[13]. **Figure 1** shows the aminolysis of ECH with DFBA and Fe^{3+} as catalyst.

MS and MS/MS optimization

The positive electrospray spectrum of a DFBA-derivatized ECH reference standard of 2.5 µg/mL in ACN:water (50:50 v/v) is shown in **Figure 2a**. Only the *m/z* range around the protonated derivatized molecule is depicted; otherwise the excess of derivatizing agent would dominate the mass spectrum. Two relevant peaks, at *m/z* 236 and *m/z* 238, which corresponded to the $[M+H]^+$ ions with ^{35}Cl and ^{37}Cl respectively, were obtained, both optimized at a cone voltage 25 V. When *m/z* 236 was used as precursor, three product ions were observed in the MS/MS spectrum. The most abundant fragment (*m/z* 127) was optimized at 20 eV collision energy (**Figure 2b**) and corresponded to difluorobenzyl ion. Two less abundant fragments were optimized at 15 eV and corresponded to *m/z* 218 and *m/z* 92 (**Figure 2c**). The proposed fragmentation pathway is shown in **Figure 3**, which is in agreement with the fragments observed in the MS/MS spectra. Taking advantage of the one chlorine atom presence in the ECH molecule, *m/z* 238 was also used as precursor ion obtaining the three product ions expected according to the fragmentation pathway proposed (*m/z* 127, 220 and 94). In this way, more SRM transitions could be monitored increasing the reliability in the identification process. Full-acquisition and MS/MS spectra for ECH-d_5 were

consistent with the fragmentation pathway proposed in this work, because losses observed for ECH-d_5 (precursor ion *m/z* 241) confirmed the presence of the five deuterium atoms in the less abundant fragments (*m/z* 223 and 97), whereas no deuterium was present in the *m/z* 127 fragment.

The experimental MS conditions and relative abundances of the product ions are summarized in **Table 1**. In spite of its lower abundance, the transition *m/z* 236>92 was selected for quantification instead of *m/z* 236>127 due to the greater background noise of the later (**Figure 4**). The notable difference in the transitions chemical noise (see relative S/N ratios in **Table 1**) seems to be a consequence of the higher specificity of the *m/z* 92 fragment in comparison to *m/z* 127, which was originated from the derivatizing agent used.

Derivatization optimization

The derivatization procedure applied was based on Khan *et al*[13]. Initially, a sample volume of 20 mL of water and 20 µL of DFBA were fixed. Then, variables as content of catalyst, reaction time and reaction temperature were optimized using an aqueous reference standard of 1.0 µg/L. Fe^{3+}, added as $FeCl_3 \cdot 6H_2O$, was used to catalyze the ECH aminolysis. Different catalyst amounts were tested, selecting a final concentration of 0.02 mM (20 µL of 6 g/L $FeCl_3 \cdot 6H_2O$ added to 20 mL of water sample). Reaction time and temperature influence were studied carrying out experiments (n=7) for ECH at 1.0 µg/L (kept in dark for 2, 3, 4, 6, 8 hours and overnight, and at room temperature, 35 and 45 ºC). The best results in terms of sensitivity corresponded to derivatization at room temperature overnight, at 35 ºC for 6 hours, and at 45 ºC for 3 hours. However, repeatability was worse when heating at 35 ºC and 45 ºC (RSD>30%), possibly due to the faster degradation of the derivatization product. Therefore, the optimum conditions chosen for derivatization reaction were overnight and room temperature. Despite the better precision reached in this case (RSD always below 10%), the addition of ECH-d_5 as IS was necessary for more satisfactory and reproducible results.

LC optimization

Different mobile phases (mixtures of water with MeOH or ACN as organic modifiers) adding different amounts of additives (NH_4Ac and HCOOH) were tested using four analytical columns with different retention mechanisms and/or particle size (Atlantis 5 µm, Discovery 5 µm, SunFire 5 µm and 3.5 µm). ECH-DFBA, similarly to other compounds determined in positive ionization mode, presented better ionization yield when methanol was used as organic modifier due to its protic character. Besides, more intense and narrow peaks were obtained using MeOH instead of ACN. Regarding additives, small amounts of NH_4Ac (0.1 mM) added, to both water and MeOH, resulted in better peak shape and ionization efficiency. Better peak shapes were observed for Sunfire columns, although the use of small particle size (3.5 µm) was discarded due to the pressure enhancements and worse peak shape after a few injections. Therefore, Sunfire column with a particle size of 5 µm (50 x 2.1 mm) was selected to carry out chromatographic separation.

In order to increase the sensitivity of the method, direct large volume injection (LVI) using different volume sample injection loops (250, 500 and 750 µL) was tested employing larger chromatographic columns (Atlantis 5 µm and Sunfire 3.5 µm, both 100 x 2.1 mm). No satisfactory results were obtained regarding peak shape and sensitivity objective (0.1 µ/L).

Then, on-line SPE pre-concentration was considered in order to reach the appropriate sensitivity. Three different stationary phases were tested for cartridges (PRP, C_{18} and Oasis HLB), using 50 x 2.1 mm, 5 µm Sunfire as analytical column. Better results were obtained when using C_{18} cartridges. Different sample loops were tested (500, 750 and 2500 µL) for sample loading. Adequate sensitivity to determine and confirm the presence of ECH at the LOQ objective (0.1 µg/L) was only possible when 2500 µL were injected.

It was required to filter all samples and standards prior to the SPE/LC/MS/MS analysis to preserve Fe (III) traces and other particles that could negatively affect columns filling. For this purpose, different particle-size nylon filters were tested (0.45 µm from Sigma and Scharlab, and 0.2

278 µm from Scharlab and Albet). Sigma 0.45 µm filters were chosen due to compound losses observed
279 with the other filters employed.
280
281 **Validation study**
282 Linearity of the SPE/LC/MS/MS method was satisfactory in the range 0.05 - 50 µg/L, with
283 correlation coefficients higher than 0.999 and residuals lower than 30%. Precision (repeatability)
284 and accuracy (expressed as recovery) were estimated by analyzing (n=3) different blank samples
285 spiked at two concentration levels each (0.1 and 1.0 µg/L): two DWTP, two DSW and one DW.
286 Results obtained are reported in **Table 2**. The method was found to have satisfactory precision and
287 accuracy with RSD < 20 % and recoveries between 70 and 103 % for all samples at the two spiking
288 levels. The method was also highly specific as no relevant signals were observed in the blanks at the
289 analyte's retention times. LOD of 0.03 µg/L was estimated from chromatograms at the 0.1 µg/L
290 level.
291 Considering absolute responses (without internal standard), we could evaluate matrix effects
292 in the different water samples tested, with a general trend to signal enhancement being observed in
293 some samples. Thus, a slight signal enhancement was observed in DWTP2 at 1.0 µg/L (recovery
294 130 %). In the sample DSW2, a matrix enhancement was also found leading to recoveries of 140
295 and 180 % for 0.1 and 1.0 µg/L fortification levels, respectively. In these samples the use of IS
296 calibration was mandatory for a correct quantification. In general, precision was also improved
297 when ECH-d_5 is used (see **Table 2**).
298 **Figure 5** shows the SRM chromatograms for the quantification (Q) and confirmation (q_1)
299 transitions of a HPLC-grade water blank, a reference standard and the DWTP1 sample spiked both
300 at 0.1 µg/L. It can be seen the robustness of the analyte and IS retention times as well as the good
301 sensitivity at LOQ level that allow to quantify and confirm ECH in water samples at sub-ppb levels.
302
303

Confirmation

An advantage associated with the use of tandem mass spectrometry is the possibility to acquire different SRM transitions to confirm the presence of analytes in the sample. Following EU guidelines recommendation, in order to assure analyte identification in samples analyzed, a minimum of 3 IPs are necessary[31]. This number of IPs can be obtained in a LC-MS/MS method with the acquisition of, at least, two SRM transitions. The method developed in this paper allows acquiring up to five transitions for ECH safe identification in a single run. However, due to the great differences between transitions intensity, confirmation at low levels (≤ 0.1 µg/L) could be only carried out with two out of five transitions, concretely *m/z* 236>92 for quantification and *m/z* 236>127 for confirmation, although reaching sufficient number of IPs. Anyway, these two transitions are enough to obtain the required IPs. Nevertheless, for ECH concentrations around and higher than 0.5 µg/L, confirmation of positive samples can be carried out making use of all the five transitions acquired.

The method was applied to ten water samples (three drinking water treatment plant, four distribution system water and three drinking water) from the Castellón province, but no ECH was detected. Quality control samples prepared from drinking water spiked at the two levels (0.1 and 1.0 µg/L) were included in each sample batch. Satisfactory recoveries (between 70-110%) were obtained, ensuring in this way the reliability of the method. In absence of positive samples, **Figure 6** shows SRM chromatograms for all transitions corresponding to a 1.0 µg/L standard and to the sample DSW1 fortified at the same concentration**.** Concentration ratios, calculated from the ECH concentrations obtained for every confirmation transition and from that calculated for the quantification transition, are also shown for the DSW sample (**Figure 6b**). All Q/q ratios were in the range 0.85 - 1.09. So, maximum deviations were ≤ 15 %, which allows a reliable and safe confirmation of ECH in samples[31].

CONCLUSIONS

Determination of epichlorohydrin in water at sub-ppb levels is rather problematic due to its highly polar character and low molecular size. This forces to apply a derivatization step when using both liquid and gas chromatography, although GC-based methods typically require more sample manipulation to make compatible the analyte with the chromatographic requirements and to reach the sensitivity required.

In this paper, we have developed sensitive, selective and accurate methodology based on a rapid on-line SPE/LC coupled to MS/MS (ESI) preceded by a simple derivatization step with DFBA, able to determine ECH in water at low concentrations. The optimized method was validated at 0.1 and 1 µg/L levels in different types of water samples, reaching limits of detection of 0.03 µg/L. The use of isotope-labelled ECH-d_5 as internal standard leads to a reliable quantification, minimizing potential analytical errors along the derivatization process, as well as instrumental deviations, also allowing compensating matrix effects that may negatively affect to quantification in LC/MS/MS-based methods.

The acquisition of up to five specific MS/MS SRM transitions together with the evaluation of their intensity ratios, gives a high degree of reliability to the identification of ECH in water samples, minimizing the risk of reporting false positives.

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405 **Table 1.** MS/MS optimized conditions for the determination of epichlorohydrin.

406

Compound	Precursor ion (*m/z*)	Cone voltage (V)	Product ion (*m/z*)	Collision energy (eV)	Relative abundance	Relative S/N ratios
ECH-DFBA	236	25	92 (*Q*)	15	3	100
			127 (q_1)	20	100	23
			218 (q_2)	15	5	13
	238		94 (q_3)	15	1	25
			127 (q_4)	20	30	20
ECH-d_5-DFBA	241	25	97 (*Q*)	15	3	75
			127 (*q*)	20	100	100

407

408 (Q) - Quantification transition, (q) – confirmation transition.

409

410 **Table 2.** Average recoveries and relative standard deviations for the SPE/LC/MS/MS method
411 applied to five different water samples spiked with ECH at two levels (n=3).
412

	0.1 µg/L		1.0 µg/L	
Sample	**Recovery (%)**	**RSD (%)**	**Recovery (%)**	**RSD (%)**
DWTP1	70	12	95	6
DWTP2	77	9	94	5
DSW 1	86	7	98	5
DSW2	80	20	102	2
DW	103	14	102	5

413
414 DWTP, drinking water treatment plant; DSW, distribution system water; DW, drinking water.
415

FIGURE CAPTIONS

Figure 1. Aminolysis of epichlorohydrin with DFBA and Fe(III) acting as a catalyst.

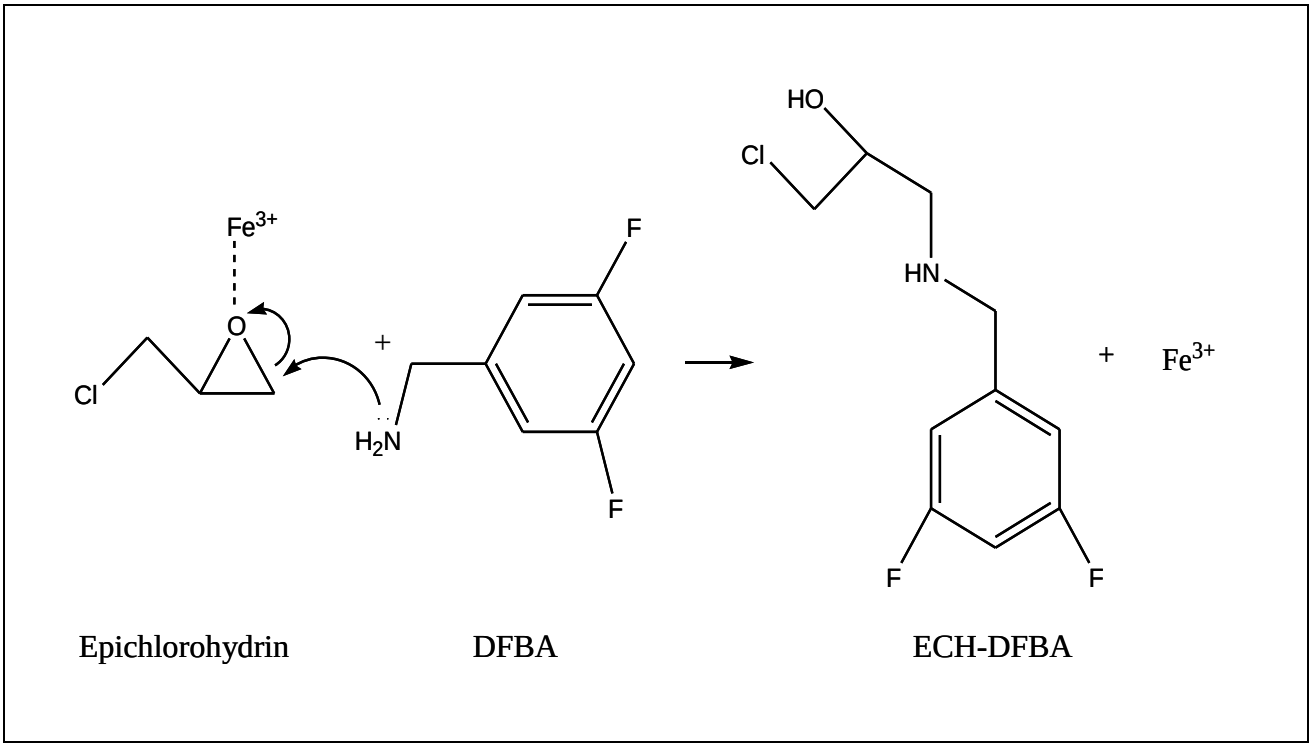
Figure 2. (a) Positive ESI mass spectrum of derivatized ECH-DFBA, cone voltage 25 V (b) Product ion spectrum for *m/z* 236 at 20 eV and (c) at 15 eV.

Figure 3. Fragmentation pathway proposed for the $[M+H]^+$ ion of ECH–DFBA.

Figure 4. Background noise in SRM chromatograms for a 2500 µL injection of 0.05 µg/L derivatized ECH reference standard. (q_1): 236>127; (Q): 236>92.

Figure 5. LC/MS/MS SRM chromatograms for derivatized ECH and ECH-d_5 (a) HPLC-grade water blank (b) Spiked DWTP1 sample at 0.1 µg/L (c) Reference standard in water at 0.1 µg/L. Top: ECH-d_5 chromatograms. Bottom: ECH chromatograms.

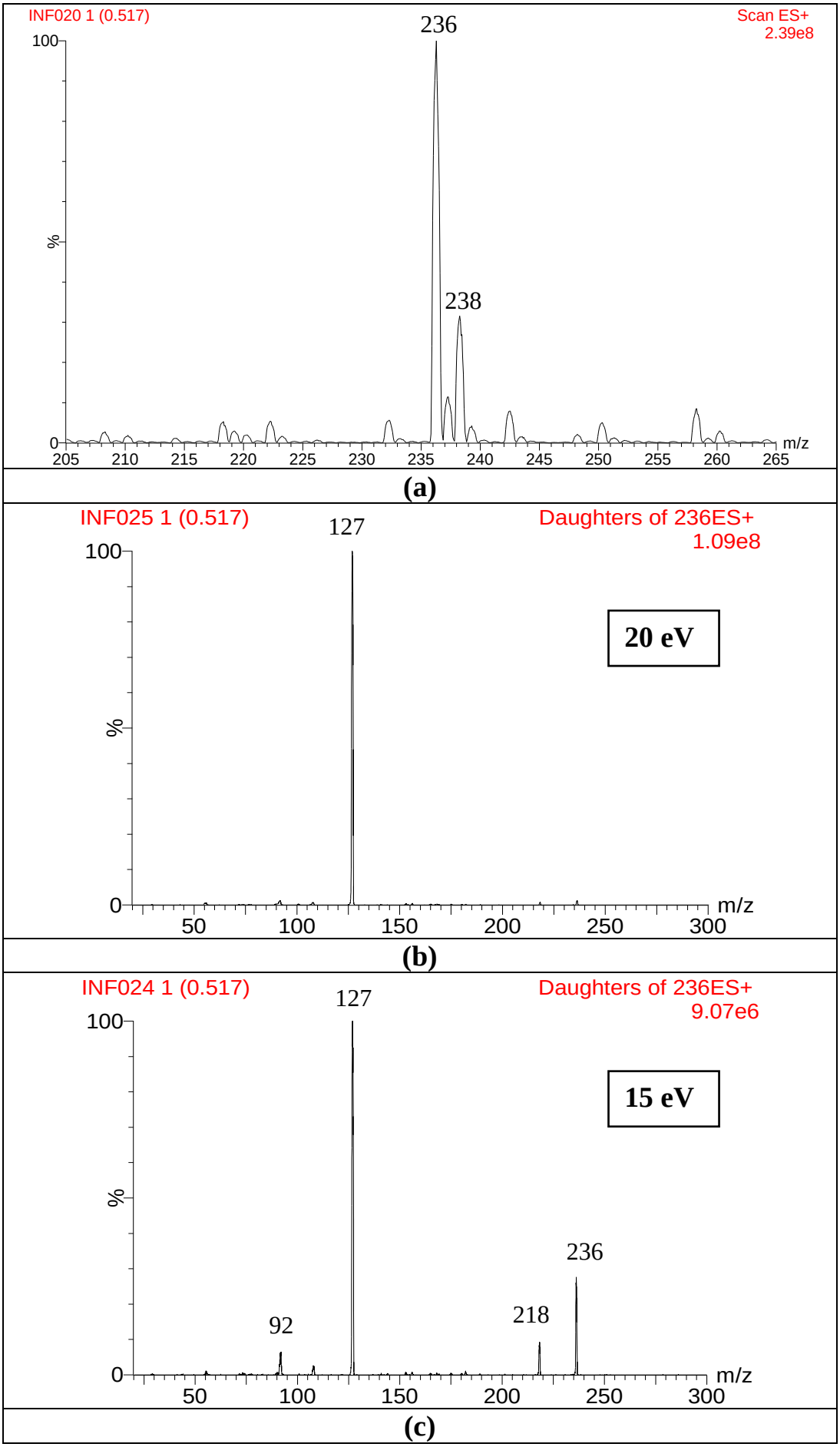
Figure 6. SRM chromatograms for all the selected transitions of (a) ECH reference standard and (b) spiked DSW1 sample, both at 1.0 µg/L.



435

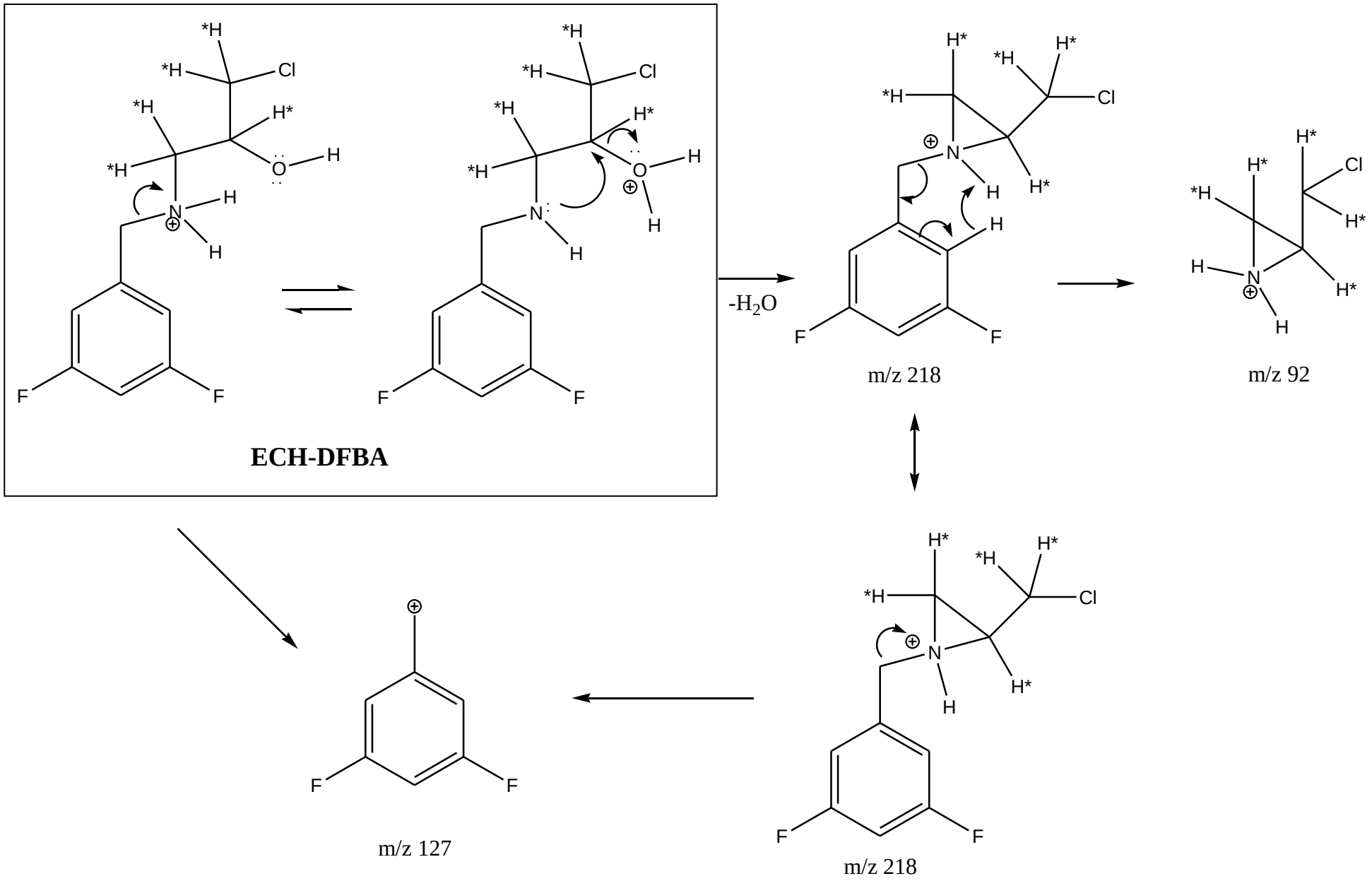
436 **Figure 1**

437



438

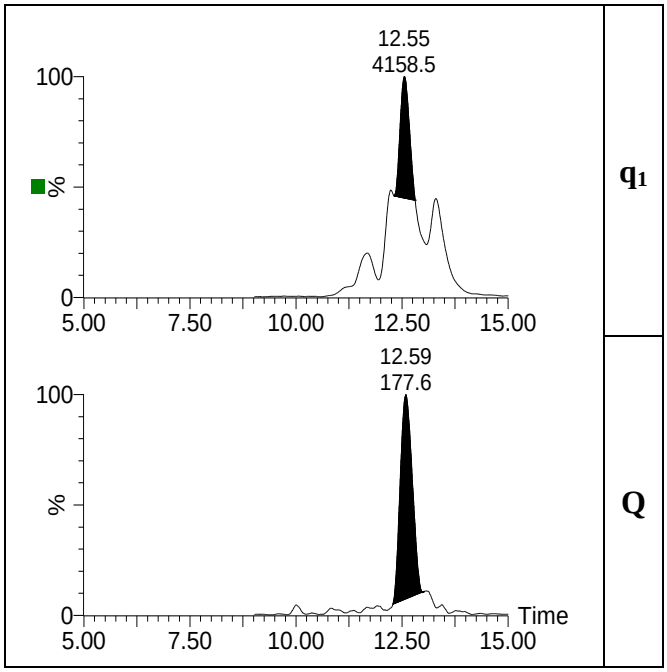
439 **Figure 2**



H*: ^{1}H in ECH-DFBA molecule; ^{2}H in ECH-d_5-DFBA molecule.

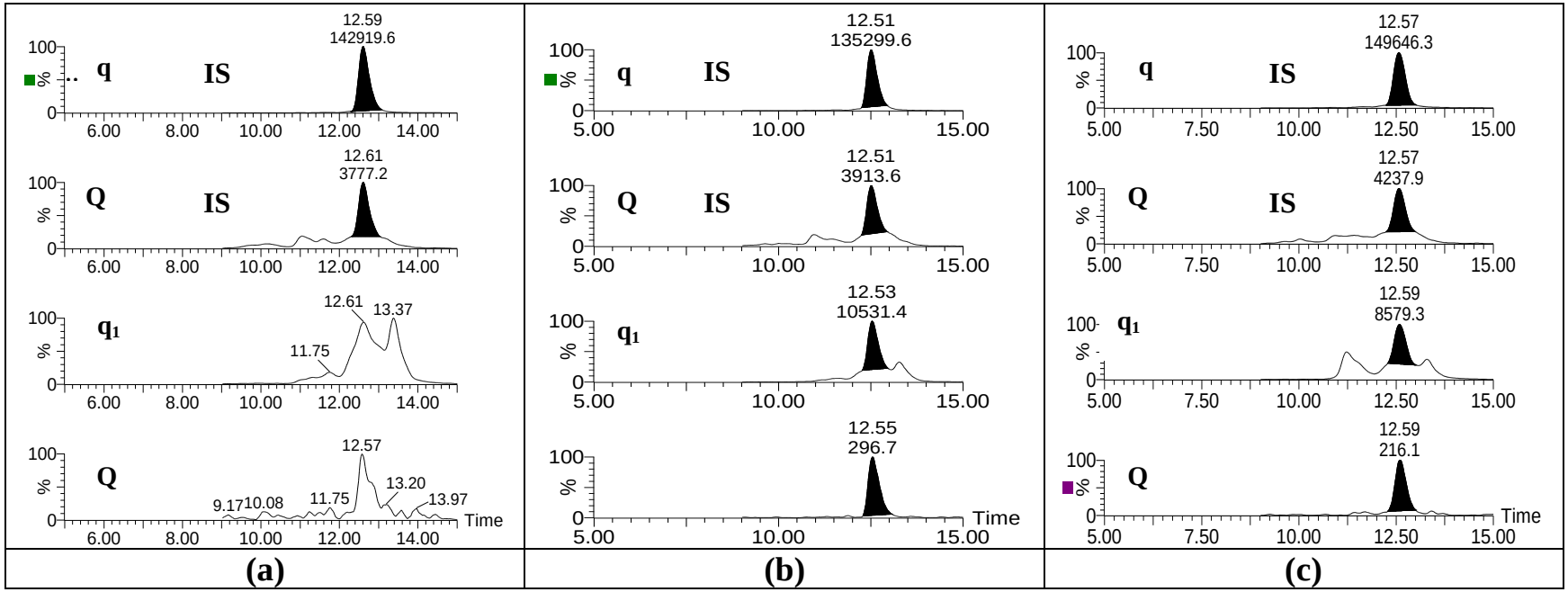
Figure 3

443



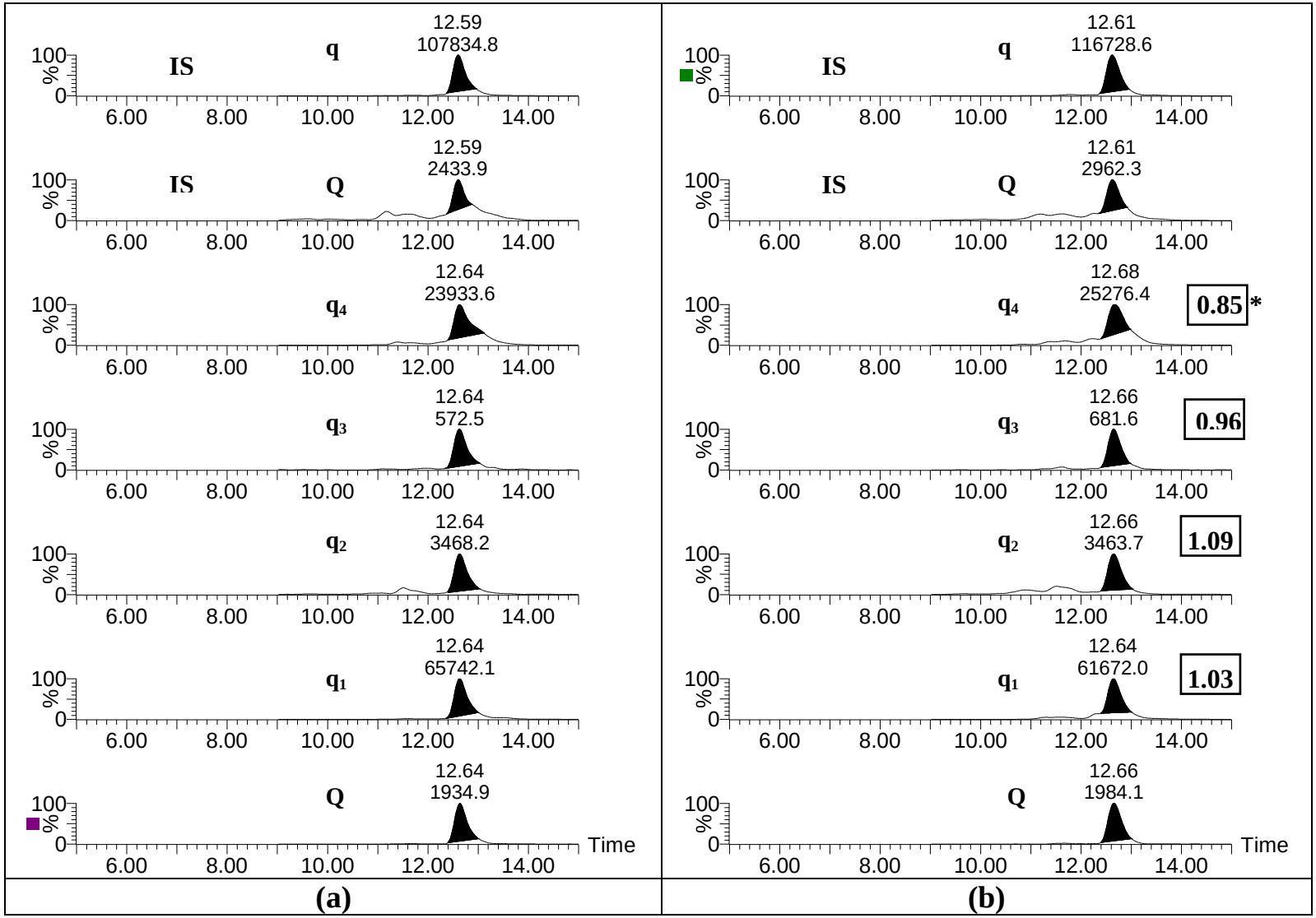
444

445 **Figure 4**



(Q) - Quantification transition, (q) – confirmation transition.

Figure 5



* Q/q Concentration ratios.
(Q) - Quantification transition, (q) – confirmation transition.

Figure 6