Talanta 126 (2014) 170–176

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Stable isotopic internal standard correction for quantitative analysis of hydroxyeicosatetraenoic acids (HETEs) in serum by on-line SPE–LC–MS/MS in selected reaction monitoring mode

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ARTICLE INFO

Article history:
Received 8 October 2013
Received in revised form
7 March 2014
Accepted 17 March 2014
Available online 1 April 2014

Keywords:
Internal standard
Stable isotope
LC–MS/MS
On-line SPE
HETE

ABSTRACT

The influence of the inclusion of a stable isotopic labeled internal standard (SIL-IS) on the quantitative analysis of hydroxyeicosatetranoic acids (HETEs) in human serum is evaluated in this research. A solid-phase extraction–liquid chromatography–tandem mass spectrometry (SPE–LC–MS/MS) platform, one of the preferred approaches for targeted analysis of biofluids through the selected reaction monitoring (SRM) operational mode, was used to determine HETEs. These compounds were chosen as targeted metabolites because of their involvement in cardiovascular disease, cancer and osteoporosis. 15HETE-d8 was chosen as internal standard to evaluate matrix effects. Thus, the physico-chemical properties of the SIL-IS were the basis to evaluate the analytical features of the method for each metabolite through four calibration models. Two of the models were built with standard solutions at different concentration levels, but one of the calibration sets was spiked with an internal standard (IS). The other two models were built with the serum pool from osteoporotic patients, which was spiked at different concentrations with the target analytes. In this case, one of the serum calibration sets was also spiked with the IS. The study shows that the IS allowed noticeable correction of matrix effects for some HETE isomers at certain concentration levels, while accuracy was decreased at low concentration (15 ng/mL) of them. Therefore, characterization of the method has been properly completed at different concentration levels.

1. Introduction

Liquid chromatography coupled to mass spectrometry (LC–MS/MS) using a triple quadrupole spectrometer (QqQ) is considered one of the best options for targeted analysis in biofluids. Methods based on selected reaction monitoring (SRM) ensures unrivaled sensitivity and selectivity both in metabolomics and proteomics studies [1,2]. One important benefit associated to the SRM mode is robustness, mainly limited by potential decreased precision caused by ionization suppression effects, particularly in electrospray ionization (ESI), which is the most widely used ionization mode. Ionization suppression may be caused by high concentration of endogenous matrix components that co-elute with the target analytes [3]. Matrix effects can be eliminated or minimized by using a highly pure internal standard (IS) [4–6] with physico-chemical properties similar to the target analyte and generating a response signal distinguishable from that of the analyte of interest. Apart from ionization suppression effects, selection of a suitable IS compensates for other variability sources induced by sample preparation steps such as dilution, concentration by solvent evaporation and/or analytes adsorption, instrumental variations including injection volume as well as variations in response over time [7,8]. In fact, the ideal IS should behave as the target analytes during the entire analytical protocol [3].

There are two different approaches in dealing with the selection of the best IS: a structural or chemical analog related to the analyte or a stable isotopic labeled internal standard (SIL-IS), which contains stable isotopes such as ^{13}C, ^{15}N, ^{18}O or ^{2}H in its molecular structure [9]. SIL-forms of a given analyte are presently the most commonly used IS in small-molecule bioanalysis using LC–MS/MS [10]. Although ^{2}H is the cheapest and most frequently used isotope, other stable isotopes such as ^{13}C, ^{15}N or ^{18}O may be more suitable since deuterium and hydrogen have greater differences in their physical properties than, for example, ^{12}C and ^{13}C [11,12]. However, the number of studies based on the use of non-deuterated IS is lower than those involving deuterated IS for

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http://dx.doi.org/10.1016/j.talanta.2014.03.038
0039-9140/

targeted analysis of human biological samples by LC–MS/MS [13,14].

The complexity in the analysis of biological samples arises from multicomponent methods, in which a number of labeled IS similar to the target compounds would be required to avoid ionization suppression effects ascribed to certain chemical structures [15]. However, these IS are not always feasible owing to both the high cost of the labeled compounds and the number of analytes to be determined, which can encompass 10, 50 or even 100 [16]. Theoretically, ionization suppression or enhancement effects caused by co-eluted matrix components should affect equally to the SIL-IS and analyte. It is, however, well-known that partial chromatographic separation of the analyte and its corresponding deuterated IS often occurs due to a small change in lipophilicity when exchanging hydrogen by deuterium [17]. How noticeable the effect is depends on factors such as the number of substituted atoms, size of the molecule, efficiency of the column, retention mechanisms and retention time. As an example, Wang et al. have shown that the MS response of carvedilol was significantly more affected than that of the SIL–IS carvedilol when they co-eluted within a region with severe ion suppression [11]: the accuracy and precision of the method were severely affected as a result.

An alternative to the use of IS to overcome matrix effects is supported on implementation of advanced sample preparation methods [18,19] with capability to suppress the contribution of matrix effects. In this sense, automated solid-phase extraction (SPE) has proved a high efficiency as high accuracy and precision can be achieved under optimum operation conditions. A number of studies involving on-line SPE coupled to LC–MS/MS analysis have been reported for determination of different families of compounds [20]. In this research, the differences found by using or not an SIL-IS in SPE–LC–MS/MS for quantitative analysis of hydroxyeicosatetraenoic acids (HETEs) in human serum are revealed. The target metabolites were 8, 11, 12 and 15 HETEs, while the selected IS was a stable isotopic hydroxyeicosatetraenoic acid (HETE) isomer. Thus, the physico-chemical properties of the IS were the basis for evaluation of the analytical features of the method for each metabolite.

2. Materials and methods

2.1. Reagents

Deionized water (18 mΩ cm) from a Millipore Milli-Q water purification system was used to prepare all aqueous solutions. The standards used—all from Cayman Chemicals (Ann Arbor, MI, USA)—were hydroxyeicosatetraenoic acid isomers 8HETE (8-hydroxy-5Z,9E,11Z,14Z-eicosatetraenoic acid), 11HETE (11-hydroxy-5Z,8Z,12E, 14Z-eicosatetraenoic acid) 12HETE (12-hydroxy-5Z,8Z,10E,14Z-eicosatetraenoic acid) and 15HETE (15-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid). 15HETE-d8 (eight deuterium atoms at 5, 6, 8, 9, 11, 12, 14, and 15 positions) was used as IS for quantitative analysis of the target analytes. Formic acid and acetonitrile (ACN) from Scharlab (Barcelona, Spain) were used for chromatographic separation. All chemicals were LC grade and used without further purification.

2.2. Blood extraction and serum isolation

Venous blood was collected in evacuated sterile serum tubes without additives (Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA) from healthy donors and post-menopausal women diagnosed with osteoporosis. Blood samples were incubated for 30 min at room temperature to allow coagulation, then centrifuged at 2000*g* for 15 min at 4 °C to isolate the serum fraction, and processed within 2 h after collection. Serum was placed in plastic ware tubes and stored at −80 °C until analysis. All steps from blood extraction to analysis were performed in compliance with the guidelines dictated by the World Medical Association Declaration of Helsinki (2004), which were supervised by the Ethical Review Board (ERB) of Reina Sofía Hospital (Córdoba, Spain) that approved the experiments. Individuals selected for this study were informed to obtain consent prior to sample extraction.

A serum pool was prepared with serum isolated from osteoporotic women for development of the calibration models and validation steps. Aliquots from this pool were spiked with the target metabolites at three concentrations (15, 35 and 55 ng/mL) according to the normal serum content described in the Human Metabolome Database (www.hmdb.ca). Non-spiked aliquots were also analyzed to elucidate the natural content of these metabolites in the original pool. The samples from healthy donors were subjected to the protocol as independent samples.

2.3. Instruments

SPE was performed with an automated workstation Symbiosis-Pharma (Spark Holland, Emmen, The Netherlands) equipped with an autosampler Reliance (Spark Holland) furnished with an 100 μL sample loop and a refrigerated stacker sample compartment. Supplementary Fig. 1 shows the scheme of the different units forming the instrumental configuration. The SPE workstation is endowed with a unit for SPE cartridges exchange—automatic cartridge exchanger (ACE) and two high-pressure dispensers (HPD) for SPE solvents delivery. Peek tubes of 0.25 mm i.d. (VICI, Houston, Texas, USA) were used for all connections. The ACE unit included two clamp valves. The Sparklink 3.10 SP3 software was used to control the system. Polaris C18-A cartridges (8 μm particle size, 10 × 2.0 mm^2, Agilent) were used as sorbent material in the SPE step. Chromatographic separation was performed by an Agilent (Palo Alto, CA, USA) 1200 Series chromatograph equipped with a Mediterranea Sea C18 analytical column (3 μm particle size, 150 mm × 4.6 mm, Teknokroma, Barcelona, Spain) thermostated at 25 °C. Detection was carried out by an Agilent 6460 triple quadrupole mass spectrometer equipped with a Jet Stream Technology electrospray ion source.

2.4. Analytical protocol for determination of the target analytes

One hundred microliters of serum 1:1 diluted with water and spiked with 30 ng/mL 15HETE-d8 as internal standard was injected in the analytical system. Then, the SPE–LC–MS/MS arrangement developed the following sequence of automatic operations. The sample preparation process started with solvation of the SPE sorbent with methanol (4 mL), conditioning and equilibration with water containing 0.01% formic acid (3 mL). Then, the sample was loaded into the cartridge with water containing 0.01% formic acid (2 mL). Under these conditions, the target compounds were retained in the sorbent, which was washed with 20% acetonitrile aqueous solution (1.4 mL) to remove interferents. The chromatographic step started by switching the left clamp valve to elute the retained analytes into the chromatographic column for 3 min. The gradient used for LC separation of the compounds increased from 40% acetonitrile (maintained for 11 min) to 80% of acetonitrile in 1 min (maintained for 8 min). A 10 min post-time was necessary to equilibrate the column prior to the next injection.

Mass spectrometry detection was performed in negative ionization mode with an electrospray source (ESI) set at 4 kV capillary voltage, 350 °C source temperature and 40 psi pressure nebulizer. Nitrogen as dessolvation gas was flowed at 10 mL/min. The target eicosanoids were monitored in the selected reaction monitoring

mode (SRM) for highly-selective and sensitive determination in a complex matrix such as serum.

The entire analytical process was completed within 33 min, while chomatographic separation was completed in 20 min. Therefore, the automated approach enabled chromatographic analysis of one sample overlapped with preparation of the next sample.

2.5. Quantitative analysis

Stock standard solutions of 15HETE, 11HETE, 8HETE and 12HETE (500 ng/mL) were prepared in ethanol and stored at −20 °C. Ten calibration levels were prepared by both diluting standard solutions and spiking human serum with standard solutions at known concentrations. The concentration of target analytes ranged from 0.125 to 100 ng/mL. 15HETE-d8 (at 30 ng/mL) was used as IS to obtain the calibration model for HETEs. The calibration curves were obtained by plotting the ratios of peak area of HETEs to that of IS, and subtracting the natural content of HETEs in serum. The calibration plots of HETEs were used for quantitation.

3. Results and discussion

3.1. Preliminary aspects

HETEs were chosen for their biochemical interest since these metabolites are obtained by oxidation of polyunsaturated fatty acids (mainly 20-carbon fatty acids) which play a regulation role in inflammation [21] and, therefore, they have proved to be involved in different pathological states such as cardiovascular diseases [22], certain types of cancer [23] or osteoporosis [24]. Taking into account the relationship between the inflammatory response and osteoporosis, a serum pool prepared from osteoporotic patients was used in this study to check the suitability of the internal standard for quantitative analysis of HETEs. This evaluation was supported on the analytical features estimated for each metabolite.

Optimum MS parameters such as voltage for efficient filtration of precursor ions in the first quadrupole, collision energy to fragment precursor ions by collision-induced dissociation (CID) and product ions selected as quantitation for each analyte are listed in Table 1. There was a unique transition with detection capability for each HETE. However, this transition was highly selective for each isomer because the fragmentation occurred differently for each of them. As Fig. 1 shows, fragmentation of precursor ions for each HETE isomer depended on the position of the hydroxyl substituent, which enabled to differentiate the isomers by the SRM transition.

3.2. Optimization of SPE and on-line elution to the liquid chromatograph

The complexity of biological samples demands for suitable sample preparation protocols able to remove potential interferents prior to chromatographic separation. The main limitation of previously reported methods involving extraction of eicosanoids from biological samples is the use of manual protocols. Generally, these protocols start with a liquid–liquid extraction (LLE) step, preconcentration by off-line SPE or simple evaporation and, finally, reconstitution prior to chromatographic analysis. These off-line approaches usually involve analyte losses, degradation of intermediate metabolites and time consuming protocols. Two extraction procedures such as LLE and off-line SPE have already been evaluated in a research for analysis of eighteen eicosanoids in human and monkey plasma [25]. Overally, the off-line SPE method yielded better recovery (> 55%) and reproducibility (< 21%) than the LLE method and, for this reason, SPE seems to be more suited than LLE. However, automation of the SPE step could improve the analytical properties of the method with on-line separation and

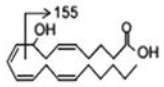
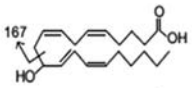
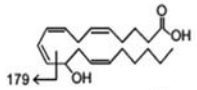
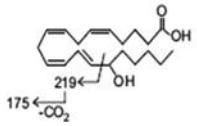
Compound	Precursor (m/z)	Product (m/z)	Structure
8HETE	319.2	155.2	
11HETE	319.2	167.2	
12HETE	319.2	179.2	
15HETE	319.2	175.2	

Fig. 1. HETEs structure with the corresponding transition.

Table 2
Optimization of the main variables involved in the SPE step.

Variable	Range tested	Optimum value
SPE sorbent	Hysphere CN, C2, C8 (EC), C18 HD, Polaris C18-A, Resin GP and Resin SH	Polaris C18-A
Sample dilution	1:1–1:5	1:1
Loading solvent		
Organic composition	From 0% to 50% of methanol and acetonitrile	0%
Acidification	From 0% to 10% of formic acid and acetic acid	0.01% formic acid
Volume	0.5–3 mL	2
Flow rate	0.2–3 mL/min	0.5
Washing solvent		
Organic composition	From 0% to 50% of methanol and acetonitrile	20% acetonitrile
Acidification	From 0% to 10% of formic acid	0% formic acid
Volume	0–3 mL	1.4
Flow rate	0.2–3 mL/min	1.3
Elution time	0.5–3.5 min	3 min

Table 1
Optimal values of the parameters involved in quantitation of HETEs by SRM mode.

Analyte	t_r (min)	Quantitation transition	Qualification transition	Collision energy (eV)	Dwell time (ms)	Voltage MS1 (eV)
15(S)HETE-d8	16.8	327.2→182.2	327.2→264.2	15	55	95
15HETE	16.9	319.2→175.2		10	55	80
11HETE	17.2	319.2→167.2		15	55	80
8HETE	17.4	319.2→155.2		10	55	80
12HETE	17.6	319.2→179.2		10	55	80

determination by LC–MS/MS. On-line SPE–LC–MS/MS was optimized in this research and an IS was used to evaluate the benefits, if present, of its implementation. Thus, the influence of the SPE sorbent, loading solvent, washing solvent and elution time—directly related to analytes retention, interferences removal and recovery, respectively—were studied by a univariate approach due to their discontinuous character. Additionally, loading and washing volumes and flow rate were set by a multivariate response surface design consisting of 16 experiments and 2 central points. Table 2 summarizes the variables optimized, the range studied and the optimum values.

3.3. Calibration models

Four calibration models were developed under the optimum working conditions to evaluate the performance and stability of the complete approach for quantitation of HETEs. Two of the models were built with standard solutions, with and without spiking the calibration solutions with the IS. The other two models were built with the serum pool from osteoporotic patients, which was spiked at different concentrations with the target analytes ($n=10$ calibration levels), also with and without spiking with the IS. Non-spiked aliquots of the same pool were also analyzed to obtain the natural content of each analyte in the serum pool, which was subtracted for preparation of the calibration curves. The results from these four calibration models are shown in Table 3 (the calibration equations and regression coefficients, R^2), and Fig. 2 (the calibration curves). The slopes, intercepts and regression coefficients of the calibration curves prepared with standard solutions were characterized by a good fitting. However, strong matrix effects were detected in the calibration models prepared with serum when their intercepts were compared with those obtained by standard solutions. This phenomenon was particularly evident in the calibration models without IS normalization. As Table 3 shows, the ratio of slopes from calibration equations in serum and standard solutions approached to the theoretical value (1.0) when the IS was considered. The no inclusion of IS in the calibration model led to critical differences between the use of standard solutions and serum, which could be clearly attributed to matrix effects. Therefore, the similarity of calibration models prepared with standard solutions and serum was higher when IS was used as compared to its no inclusion. This fact is indicative of a noticeable correction effect thanks to the IS. The similarity between both calibration models with spiked IS was as follows: 8HETE (ratio 0.98), 11HETE (1.06), 15HETE (1.12) and 12HETE (1.31). The correction effect, estimated by subtracting the ratios obtained with and without IS, was as follows: 12HETE (0.21), 15HETE (0.19), 11HETE (0.14) and 8HETE (0.13). The subtraction of the ratios showed a higher IS correction for 15HETE and 12HETE than for 8HETE and 11HETE. This high correction effect could be explained by chemical similarity to the IS, 15HETE-d8, since the hydroxyl group is farther from position 15 in 11HETE and 8HETE metabolites (see Fig. 1).

3.4. Validation of the models

3.4.1. SPE efficiency

The efficiency of the SPE step was evaluated with a dual cartridge configuration based on the in-serial coupling of two cartridges through which the sample was sequentially circulated helped by the loading solution. Supplementary Fig. 2 shows the modified scheme for this configuration. In this way, the analytes non-retained in the first cartridge could be retained in the second to estimate the retention efficiency. Then, the material retained in both cartridges was sequentially eluted to the analytical column to compare the results obtained from both cartridges and, thus, to evaluate the efficiency of the SPE step.

This study was carried out with a serum pool spiked with the target analytes at three concentrations (15, 35 and 55 ng/mL). Fig. 3 shows the retention efficiencies, expressed as percentage, obtained for each analyte as a function of the spiked concentration, with values above 90.0% for all target analytes, which is indicative of an efficient retention in the first cartridge. The values of relative standard deviation were below 2.0%; therefore, the SPE protocol provided high retention efficiency for the target compounds in the first cartridge.

3.4.2. Sensitivity

The sensitivity of the method was evaluated by estimation of the detection and quantitation limits both with standard solutions and serum samples spiked at low concentrations. This analytical feature was assessed by estimation of the limits of detection (LOD) and quantitation (LOQ) for the analytes by injecting dilution series of each in serum to obtain the concentrations which provided signals three and ten times the background noise, respectively. As shows Table 4, the LODs in the calibration model prepared with spiked serum ranged between 75 and 151 pg/mL, while the LOQs were from 0.25 to 0.5 ng/mL. On the other hand, the sensitivity was clearly improved when standard solutions were used to prepare the calibration model, except for 15HETE, as shown in Table 4. Matrix effects justify the loss of sensitivity, which was especially relevant for 12HETE. This compound was the last eluted from the analytical column (17.6 min) and, therefore, the most seriously affected by ionization suppression effects owing to lipophilic substances. Comparison with previous methods based on off-line SPE for the same analytes revealed an improvement in sensitivity terms by using the on-line configuration. In fact, LODs (from 0.075 to 0.151 ng/mL) were

Table 3
Parameters of the calibration models prepared with standard solutions and a serum pool.

Analyte	t_r		Standard solutions		Serum		Slope	
			Calibration equation	Regression coefficient R2	Calibration equation	Regression coefficient R2	Ration (serum slope/ standard solution slope)	Ratios subtraction
15HETE	16.9	With IS	$y=0.0568x+0.0109$	0.999	$y=0.0638x+1.0475$	0.993	1.12	0.19
		Without IS	$y=450.54x-562.28$	0.997	$y=590.94x+6403.6$	0.995	1.31	
11HETE	17.2	With IS	$y=0.2221x+0.1338$	0.996	$y=0.2366x+4.5293$	0.985	1.06	0.14
		Without IS	$y=1775.7x-21.197$	0.998	$y=2145.2x+38048$	0.993	1.20	
8HETE	17.4	With IS	$y=0.063x+0.0463$	0.996	$y=0.062x+1.2301$	0.995	0.98	0.13
		Without IS	$y=503.54x+96.339$	0.998	$y=561.32x+10434$	0.998	1.11	
12HETE	17.6	With IS	$y=0.0767x+0.0464$	0.996	$y=0.1008x+3.6031$	0.990	1.31	0.21
		Without IS	$y=610.69x+398.6$	0.995	$y=930.45x+33850$	0.993	1.52	

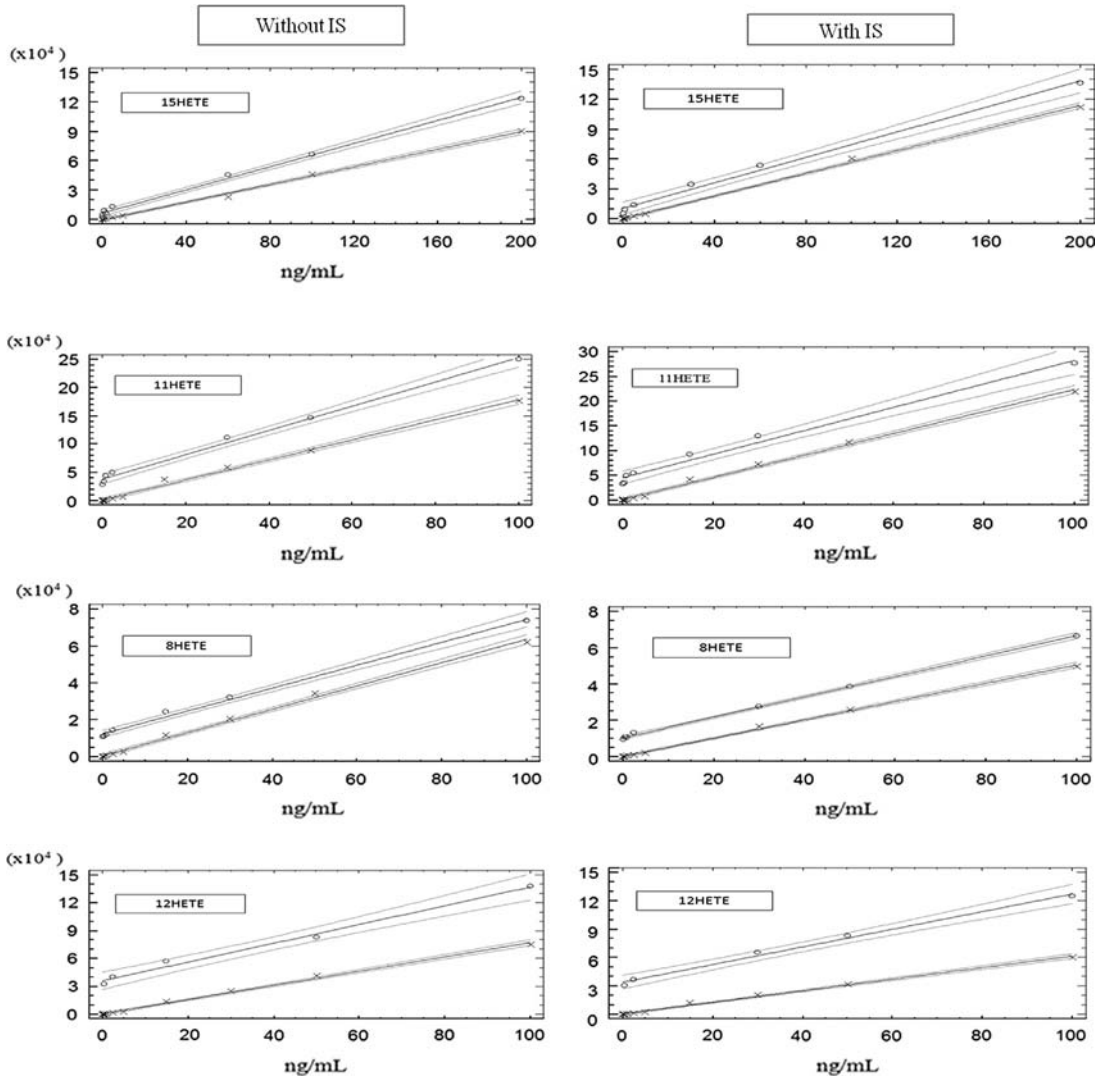


Fig. 2. Calibration curves obtained for HETES using standard solutions and spiked serum with and without IS. (X=Standard solutions, O=Serum).

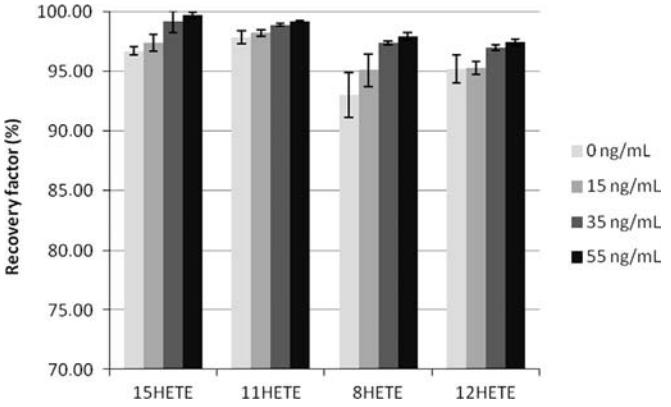


Fig. 3. Recovery for each analyte calculated with a two-cartridge configuration.

lower than those obtained by methods based on off-line SPE (range from 0.084 to 0.448 ng/mL) [26].

3.4.3. Precision

The precision of the method was evaluated by an experimental strategy intended to estimate intra-day variability (by repetitive analyses within the same day) and inter-days variability (by analyses for three consecutive days), as recommended by the Food and Drug Administration (FDA) [27]. The study was carried out with serum aliquots spiked at three concentrations and analyzed in triplicate. Precision, calculated as relative standard deviation (RSD) and expressed as percentage, was evaluated with and without internal standard correction. Table 5 shows the values obtained for the three experimental sets as a function of the spiked concentration. While no substantial effects on intra-day variability were observed by IS correction when added at 15 or 35 ng/mL, an increased variability occurred when the IS was at 55 ng/mL. Inter-days variability was clearly improved when the IS

Table 4
Limits of detection and quantitation for each analyte (expressed as pg/mL) obtained by the Calibration models prepared with standard solutions and serum.

Analyte	Standard solutions		Serum	
	LODs	LOQs	LODs	LOQs
15HETE	75	250	75	250
11HETE	38	125	75	250
8HETE	38	125	75	250
12HETE	38	125	151	250

Table 5
Intra-day and inter-day variability for each analyte in serum (at three different concentrations), expressed as % RSD, with and without IS.

Analyte	Intra-day variability (%)						Inter-day variability (%)					
	15 ng/mL		35 ng/mL		55 ng/mL		15 ng/mL		35 ng/mL		55 ng/mL	
	Without IS	With IS	Without IS	With IS	Without IS	With IS	Without IS	With IS	Without IS	With IS	Without IS	With IS
15HETE	4.2	4.1	1.3	2.3	3.6	5.7	13.6	7.0	5.7	6.3	3.8	8.4
11HETE	7.9	7.2	2.9	2.7	2.6	4.1	14.9	7.6	7.2	5.7	3.5	7.4
8HETE	2.4	2.9	7.7	5.9	2.6	1.0	12.7	5.2	8.9	5.9	4.9	7.4
12HETE	5.3	4.4	1.8	2.9	3.1	3.0	9.8	5.9	4.5	4.3	3.1	4.9

Table 6
Accuracy estimation for each HETE usin the single-cartridge configuration in serum spliked at three concentrations. The given values correspond to the recovery factor calculated for each added concentration.

Analyte		Recovery factor (%)		
		15 ng/mL	35 ng/mL	55 ng/mL
15HETE	Without IS	43.3	53.9	61.8
	With IS	73.0	85.2	83.5
11HETE	Without IS	39.4	61.4	72.1
	With IS	72.5	97.3	93.3
8HETE	Without IS	41.7	55.7	72.2
	With IS	72.6	87.7	95.5
12HETE	Without IS	54.6	50.0	63.3
	With IS	78.5	76.1	72.4

was at 15 ng/mL, slightly improved for 11HETE, 8HETE and 12HETE at 35 ng/mL IS and deteriorated at 55 ng/mL. Nevertheless, the RSDs obtained by IS correction were always below 9.0%, which is a quite acceptable value in clinical analysis.

3.4.4. Accuracy

Accuracy estimation is crucial to evaluate matrix effects in methods involving sample preparation protocols, especially those based on SPE steps. For this purpose, serum aliquots spiked at three concentrations were analyzed using the instrumental configuration based on a single cartridge. Non-spiked serum aliquots were also analyzed in this study to take into account the presence of the target metabolites in the serum pool. Accuracy was calculated by the recovery factor using serum calibration curves with and without IS. The results obtained by both models are listed in Table 6. As can be seen, the recovery factor was significantly improved when calibration models including the IS were used. In fact, the ionization suppression is significant when IS correction is not applied. For HETE isomers the recovery factors were above 83% at 35 and 55 ng/mL, except for 12HETE which yielded a 72% recovery factor at the highest spiked concentration of IS. At 15 ng/mL IS the recovery factors were below 78.5% for all HETE isomers.

With these premises, it can be said that the IS allows noticeable correction of matrix effects (viz. ionization suppression and decreased SPE retention efficiency) for some HETE isomers thus improving the analytical features of the method. Therefore, quantitation of 8HETE, 11HETE and 15HETE was properly supported on analytical parameters at medium concentrations of them (35 and 55 ng/mL), while the analysis of 12HETE was characterized by a low recovery factor at the three tested concentration levels. On the other hand, quantitation at low concentration of eicosanoids was characterized by a decrease of the recovery factors below 78.5%. Therefore, the implementation of correction factors would be required for accurate quantitation at specific concentration ranges if a given accuracy is demanded.

4. Conclusion

The effect of 15HETE-d8 as SIL-IS in the quantitative analysis of HETEs has been checked by SPE–LC–MS/MS. Calibration models prepared with serum aliquots revealed important matrix effects for HETE isomers, which were confirmed by accuracy estimation. Nevertheless, quantitative analysis of these metabolites can be assessed by application of the recovery correction factor and using an internal standard. In summary, the SIL-IS selected in this research was crucial for quantitative analysis of the target HETEs by SPE–LC–MS/MS, which demand for application of a recovery correction factor to compensate for matrix effects affecting retention of analytes in the sorbent and ionization suppression.

Acknowledgments

The Spanish Ministerio de Economía y Competitividad (MINECO) and FEDER Program are thanked for financial support through Project (CTQ2012-37428). F. Priego-Capote is also grateful to the MICINN for a Ramón y Cajal Contract (RYC-2009-03921).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.03.038.

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