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Simple and rapid method for the simultaneous determination of cholesterol and retinol in meat using normal-phase HPLC technique

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Abstract A direct saponification and normal-phase high performance liquid chromatography (NP-HPLC) procedure was developed for simultaneous determination of cholesterol and retinol in meat. A normal-phase silica column fitted with diode array detection at 208 nm for cholesterol and fluorescence detection (λ-excitation 344 nm / λ-emission 472 nm) for retinol, with mobile phase consisting of 2% (v/v) 2-propanol in n-hexane was used. Cholesterol was eluted at 10 minutes and retinol at 12.66 minutes. High linearity ($R^2 > 0.9996$ for both compounds) in calibration range was obtained. The LOD and LOQ values showing the high sensitivity of the developed methodology for simultaneous determination of cholesterol and retinol in meat. Recovery results obtained in this study (98.67-102.14% for cholesterol and 91.72-98.27% for retinol) were between AOAC recommendations to validated method and were comparable to most recent studies in precision and accuracy. In addition, the present method showed high repeatability and reproducibility. As a general conclusion, the results indicate that the direct saponification, extraction and HPLC analysis is an adequate method for cholesterol and retinol analysis in meat samples.

Keywords Food analysis; Cholesterol; Vitamin A; Meat; Method validation; HPLC-PDA-FL

Introduction

Nowadays, the high consumption and demand of meat makes necessary the development of new tools to assess the meat quality. Cholesterol, fat content and fat composition of meat are important health issues for consumers because they have been associated with obesity, hypercholesterolaemia and cancer (Chizzolini et al. 1999). Therefore, the quantification of total cholesterol and vitamin A could provide valuable information relating to meat quality.

Few methods for cholesterol determination have been validated on a performance basis as recommended by AOAC, making it difficult for commercial laboratories to adopt such methods for routine analysis. However, this method is still complicated, using large amount of chemicals for post-extraction of unsaponified materials, and includes many other preparation steps such as drying, reconstitution, and derivatization of cholesterol prior to gas chromatographic analysis (AOAC 1996a; b). Moreover, most cholesterol and retinol determination methods used with meat samples have not been validated for use on such a complex matrix with a high protein content, variable fat content, and abundance of cholesterol tightly bound in cell membranes, and the analytical methodology has been claimed to be a source of differences found in number of studies on cholesterol content of meat and poultry products (Bragagnolo 2009). Therefore, for the laboratories, validation of a simple method for the simultaneous determination of these compounds in meat is a good alternative to official methods, and help standardize the results obtained in different studies.

Thus, the objective of this study was to determine the intralaboratory performance of a simultaneous cholesterol and retinol determination method that combines direct

saponification of a 2 g meat sample and HPLC-PDA-FL quantification of liberated cholesterol and retinol.

Materials and methods

Sample preparation

Two grams of meat (beef) sample were saponified with potassium hydroxide in ethanolic solution to liberate cholesterol and retinol. Unsaponified materials were extracted with n-hexane. The n-hexane extract was injected and cholesterol and retinol were quantified using HPLC-PDA-FL technique.

Reagents

2-Propanol (HPLC grade), n-hexane (HPLC grade), ethanol (analytical grade), sodium sulphate anhydrous (>99%) (analytical grade), butylated hydroxytoluene (>99%) (analytical grade), cholesterol (>99%) and all-trans-retinol (>95%) were purchased from Sigma-Aldrich (Sigma-Aldrich, Madrid, Spain) while potassium hydroxide (85%) from Merck (Merck, Madrid, Spain). A sample of beef loin was bought at a local market (moisture: 65.5±4.1%; fat: 11.7±2.4%). This meat was minced until a homogeneous sample, and frozen (-20ºC) until the following analyses were performed.

Direct saponification and extraction

Meat samples were directly saponified according to Mestre Prates et al. (2006) procedure, with the modifications proposed by Domínguez et al. (2015). Two grams of homogenized meat sample were placed in a screw teflon-lined cap tube, in duplicate, and 0.25 g of L-ascorbic acid and 5 mL of saponification solution were added. The saponification solution, freshly prepared each week, contained 11% w/v potassium hydroxide in a

84 mixture of 55% v/v absolute ethanol and 45% v/v distilled water. The air was eliminated
85 from the reaction, by displacement with nitrogen gas and the sample was shanked until
86 the ascorbic acid was completely dissolved. The samples rested 5 minutes and then
87 vortexed. The saponification was carried out in a shaking water bath (THER-SPIN, Orto
88 Alresa, Madrid, Spain) (200 rpm) at 85 ºC for 45 min. Samples were vortexed after 20
89 minutes of saponification process.
90 After saponification, samples were cooled in tap water for 10 min. Following cooling
91 1.5 ml of distilled water and 3 ml of 25 µg/ml BHT solution in n-hexane were added (final
92 proportions of 4.5 ml H_2O: 3 ml ethanol: 3 ml n-hexane; the meat sample was assumed
93 to contribute with 0.5 ml H_2O). The samples were vigorously vortexed and centrifuged at
94 1500 g for 3 min. Emulsification may occur during the extraction process. If an emulsion
95 is formed, 0.2 mL of absolute ethanol can be added to facilitate the separation process.
96 The ethanol is gently mixed in swirling motion, and the emulsifying mixture allowed to
97 stand to enable the separation. An emulsion needs to be eliminated or decreased before
98 subsequent steps are performed. An aliquot of the upper layer (n-hexane) was transferred
99 into a small screw teflon-lined cap tube and a spatletip of anhydrous sodium sulphate was
100 added. Finally, the tube was briefly shaken and an aliquot of the n-hexane was filtered
101 through a 0.45-µm hydrophobic membrane into an amber screw-cap vial with teflon
102 septum.

103 **Instrumentation and chromatographic conditions**

104 The HPLC systems used was an Alliance 2695 model (Waters, Milford, USA) equipped
105 with a 996 Photodiode Array Detector (Waters Milford, USA) and 2475 Multi-λ
106 Fluorescence Detector (Waters Milford, USA). Empower 3TM advanced software
107 (Waters, Milford, USA) was used to control system operation and results management.

The cholesterol and retinol analysis were performed using a normal-phase silica column (SunFireTM Prep Silica, 4.6 mm ID × 250 mm, 5 µm particle size, Waters, Milford, MA, USA). The solvent (2% v/v 2-propanol in n-hexane) flow rate was 1 ml/min, the run last for 15 min and the temperature of the column oven was adjusted at 30 ºC. From each standard and sample 10 µL was injected. The detection of cholesterol was carry out using Photodiode Array detector at 208 nm, while the retinol was detected using the Fluorescence detector (λ-excitation 344 nm / λ-emission 472 nm). Total cholesterol and retinol in meat samples were quantified based on the external standard technique, from a standard curve of peak area *vs.* concentration (Fig 1). Results were expressed as mg of cholesterol/100 g of meat and µg of retinol/100 g of meat.

Method validation

The developed normal-phase HPLC methodology was validated in terms of linearity, limit of detection (LOD), limit of quantification (LOQ), peak characteristics, accuracy and precision (Barba et al. 2013a; b).

Linearity was evaluated using the external standard calibration method. The stock solutions were prepared by dissolving 100 mg of the cholesterol standard and 125 µL of the all-trans-retinol solution (1000 µg/mL) in 100 mL of n-hexane to make up solutions of 1000 µg/mL of cholesterol and 1250 ng/mL of retinol. Later, they were diluted to obtain eight calibration levels ranging from 8 to 1000 µg/mL of cholesterol standard and eight calibration levels ranging from 20 to 1250 ng/mL of retinol standard. Three sets of fresh standard solution were prepared independently and were subjected to HPLC calibration on three different days (two sets/day) to determine linearity, including R^2, standard errors of both the slope and intercept. The response factors from calibration lines of peak area (A) over concentration were also calculated.

The LOD and LOQ were determined as the cholesterol and retinol concentrations corresponding to signal to noise ratios of 3 and 10, respectively (according to ICH recommendations) (ICH 2005). The baseline noise was taken as the height (AU) of the highest noise peaks in ten chromatograms of blank samples (n-hexane) at the retention time corresponding to that of free cholesterol and all-trans retinol standard.

Peak characteristics were also analysed. The retention time of standards (8 levels x 3 replicates x 2 injections) and samples (8 repetitions per day x 3 consecutive days) and peak width of standards (8 levels x 6 readings) were measured. Specificity was determined using the resolution of the peaks. The resolution was measured by the ratio of the distance between two neighbouring peak maxima and the mean of the two peak widths, according to CDER (1994) recommendations. For the peak asymmetry analysis, the tailing factor was calculated, according to CDER (1994): 10% above the baseline as the ratio of the width from peak maximum to the end and the width from peak front to the maximum.

Accuracy was evaluated as the recovery obtained when 3 different amounts of cholesterol (95, 190 and 300 µg/g) and retinol (45, 105 and 240 ng/g) standard were added to eighteen meat samples (six per level) (injected in duplicate). The recovery efficiency was calculated using ratio of cholesterol (mg/100 g) and retinol (µg/100 g) content recovered to the cholesterol and retinol content added. The calculation was carried out using the following equation for each compound:

$$R(\%) = Cr \times 100 \times \left(\frac{1}{Ca}\right)$$

Where *Cr* is the cholesterol and retinol content recovered (mg/100 g and µg/100 g for cholesterol and retinol, respectively), which was derived from subtraction of non-addition

155 concentration and *Ca* is the cholesterol and retinol content added (also expressed in
156 mg/100 g and µg/100 g for cholesterol and retinol, respectively).
157
158 **Intralaboratory precision**

159 Eight repetitions of meat sample were weighed, saponified and injected under the same
160 analytical conditions within a day (intraday). These analyses were replicated with
161 different saponification solutions (freshly prepared each replicate) in three different days
162 (interday) to analyse the variation in different days. The intralaboratory repeatability was
163 assessed using the relative standard deviation (% RSD) of the eight replicates analysed
164 on the same day (first day), whereas the 24 replicates on the three days were used for the
165 intralaboratory reproducibility. The interday precision was also evaluated with analysis
166 of variance, to determine significant differences between replicates.

167 **Statistical analysis**

168 Forty-eight injections of standards (8 levels x 3 replicates x 2 injections) and twenty-four
169 injections of meat sample (8 repetitions per day x 3 consecutive days) were analysed in
170 the present study. Experimental data analysis and the calculation of the different
171 parameters were achieved using IBM SPSS statistics for Windows, version 19.0. IBM
172 Corp., New York, NY, USA). For the statistical interday differences an analysis of
173 variance (ANOVA) of one way using the same statistical software package was
174 performed.

175 **Results and discussion**

To demonstrate the method suitability for routine identification and quantification of cholesterol and retinol in meat, the analytical characteristics of the developed HPLC-PDA-FL methodology for the determination of cholesterol and retinol were evaluated.

Peak characteristics

The retention times (RT) and peak characteristics of cholesterol and retinol for standards and samples are presented in Table 1. The mean of cholesterol RT (both in standard and samples; n=72) was 10.00±0.01 minutes, while the retinol RT was 12.66±0.01minutes. These data confirm that there were no variations in the retention times between samples and standards. Therefore, it can be stated that there is no effect of the sample matrix in the chromatographic analysis. Peak width was measured using the standard injections (all levels; n=48). Cholesterol analysis showed a mean peak width of 0.86 minutes and retinol of 0.78 minutes. In addition, the amount of cholesterol and retinol did not affect the peak width. The injections of the lowest concentration level of cholesterol standard (8 µg/mL) presented a peak with of 0.85±0.03 minutes and the highest concentration levels (1000 µg/mL) showed a peak width of 0.86±0.04 minutes (data not shown). In the same way, the injections of the lowest concentration level of retinol (20 ng/mL) presented a peak with of 0.79±0.04 minutes and the highest concentration levels (1250 ng/mL) showed a peak width of 0.82±0.02 minutes (data not shown).

Specificity is the ability to assess the component of interest without interferences from other components which may coexist due to similar chemical and physical properties (Kua et al. 2016). In chromatography, specificity is assessed by peak resolution. According to CDER (1994), if the peak resolution is greater than “2” ensures the two neighboring peaks resolve completely from each other. The specificity (assessed by peak resolution) of the meat samples (n=24) used in the present study was 15.53 for cholesterol

and 14.41 in retinol. Therefore, the resolution was >7 times greater than that recommended value for a complete resolution. In fact, the two peaks were well separated in a very clean chromatogram. It is possible to observe in the chromatogram examples (Fig. 2) for standards and samples, that there were no peaks near the cholesterol or retinol. As commented above, the Official Method of AOAC (Official Method 994.10) (AOAC 1996) is the only certified method for cholesterol determination, using gas chromatography (GC) with a flame ionization detector. In a more recent study, Dinh et al. (2012) also used GC technique to the determination of total cholesterol in meat and poultry. In a similar way, the retinol could be also determined in meat using GC techniques (Maraschiello and Regueiro 1998). However, in contrast to HPLC analysis that resolve efficiently these compounds, the use of GC, although GC columns are very efficient at cholesterol separation, usually suffer from possible overlapping of cholesterol with other sterols and especially α-tocopherol, which co-elute with cholesterol on many GC systems (Fenton 1992). The effectiveness of cholesterol analysis using normal-phase HPLC technique was also observed by Katsanidis and Addis (1999) and Mestre Prates et al. (2006), who successfully separated and quantified cholesterol than other compounds that is co-eluted with cholesterol in GC (vitamin E and its homologs) using a column (Zorbax RX-Sil column; 250 mm x 4.6 mm x 5 μm) with the same characteristics that the column used in the present study. Other possible option to cholesterol and retinol analysis is the use of reverse-phase HPLC analysis. The reverse-phase HPLC method uses a non-polar column or a column with low polarity and a polar mobile phase. Simple addition of some polar solvents such as methanol, ethanol, or water can dramatically change mobile phase polarity and alter the elution (Abidi 2001). Domínguez et al. (2015b) quantified both, cholesterol and retinol in pork meat, liver and adipose tissues using a Ultrasphere ODS (C18) (250 mm x 4.6 mm x 5 μm) with methanol:acetonitrile:water (68:28:4) as

225 mobile phase and a flow rate of 2 mL min. Dobreva et al. (2017) also determine
226 cholesterol and retinol in seaweed and mussel tissue using reverse-phase column (Synergi
227 4μ Hydro-RP 250x4.6 mm) and gradient analysis with water, methanol, acetonitrile and
228 2-propanol during 40 minutes, while López-Cervantes et al. (2006) quantified cholesterol
229 and retinol in shrimp waste hydrolysate using a SS Exil ODS column (250 mm x 4 mm x
230 5 μm) with methanol:acetonitrile:water (68:28:4) as mobile phase and a flow rate of 1.4
231 mL min. Bragagnolo and Rodriguez-Amaya (2002) and Salvatori et al. (2008) also used
232 reverse-phase HPLC techniques for the quantification of cholesterol in pork meat and
233 backfat.

234 Other important characteristic of the peak is tailing factor. This factor affects the
235 compound quantification. According to validation of chromatographic methods, accuracy
236 of quantitation decreases with increase in peak tailing because of the difficulties
237 encountered by the integrator in determining where the peak ends and hence the
238 calculation of the area under the peak. Therefore, if the integrator is unable to determine
239 exactly when an upslope or downslope occurs, accuracy drops (CDER 1994). In this
240 study, the tailing factor (both in standards and samples; n=72) was 0.19 for cholesterol
241 and 0.14 for retinol. These values are 10-14 times less than the maximum value
242 recommended by CDER (1994) (tailing factor ≤2).

243 **Linearity, LOD, LOQ and accuracy of the HPLC-PDA-FL method**

244 Linearity is the ability to obtain signal results which are directly proportional to the
245 concentration of analyte. In this study, linearity was established from eight calibration
246 levels ranging from 8 to 1000 μg/mL for cholesterol and from 20 to 1250 ng/mL for
247 retinol using commercially standards. R^2 values tabulated in Table 1 were more than
248 0.9996 indicating that the calibration lines correlated well with the data. The values

obtained (between 0.9996 and 0.9999) are higher than 0.999 recommended by Validation of Chromatographic Methods (CDER 1994). A R^2 of near to 1 ensures the best agreement with the linear range of calibration. The R^2 value for cholesterol was similar to those described for authors using normal-phase HPLC determination (R^2= 0.9981; Mestre Prates et al. (2006)) or using GC techniques (R^2= 0.995; (Dinh et al. 2012); R^2= 0.999; Meier et al. (2006)), while the value of R^2 for retinol was higher than those described by Maraschiello and Regueiro (1998) (R^2= 0.9751). The response factor (expressed as area/concentration) was 8.11 for cholesterol and 19.99 for retinol.

Limit of detection (LOD) and limit of quantification (LOQ) were determined based on the signal to noise ratio calculated as the concentration yielding an S/N ratio of 3 and 10, respectively. For cholesterol, the LOD and LOQ of the investigated method were 3.90 and 7.80 µg/mL, respectively, which allow the detection of 0.59 mg and the quantification of 1.17 mg of cholesterol per 100 gram of sample analyzed. For retinol, the LOD and LOQ of the investigated method were 6.25 and 19.55 ng/mL, respectively, which allow the detection of 0.94 µg and the quantification of 2.93 µg of retinol per 100 gram of sample. These results show the high sensitivity of the developed methodology for simultaneous determination of cholesterol and retinol in meat. In fact, the LOD and LOQ values for cholesterol were lower in this study than those described by Dinh et al. (2012) (LOD: 1.24 mg/100 g; LOQ: 3.76 mg/100 g) using GC-FID. Therefore, HPLC technique allows obtaining higher sensitivity than using GC techniques.

Accuracy is the measure of how close the experimental value is to the true value. The accuracy of the developed analytical method was assessed by evaluating the recovery of cholesterol and retinol when meat samples were spiked with three concentrations of both compounds (95, 190 and 300 µg/g of cholesterol and 45, 105 and 240 ng/g of retinol). The results of the study of percentage recovery are summarized in Table 1. The recovery

obtained for cholesterol was between 98.67 and 102.14%, while the recovery obtained for the retinol was between 91.72 and 98.27%. The recoveries obtained in the present study, for both, cholesterol and retinol are between AOAC recommendations to validated method (90% ≤ recovery ≤ 107%) (AOAC 2002). With these results in mind it is possible to conclude that direct saponification, followed by extraction and subsequent HPLC analysis is the most appropriate methodology for cholesterol and retinol analysis in meat samples. The recovery obtained by Dinh et al. (2008) for cholesterol analysis using GC-FID technique was between 92.86 and 107.22%, while in a more recent study, the same authors obtained recoveries between 77.08 and 100.56% (Dinh et al. 2012). The use of normal-phase HPLC showed a mean recovery of cholesterol of 93% (Mestre Prates et al. 2006). The recovery of retinol obtained in the present study was higher than those described by Maraschiello and Regueiro (1998) using GC-FID, who observed recoveries between 76.78% and 89.19% in spiked muscle samples.

Intralaboratory precision

Finally, precision was evaluated considering the instrumental repeatability and the intermediate precision. Method and instrumental repeatability were determined after saponification, extraction and injection of eight meat samples in day 1. This process was carried out three different days to observe de variation between days (intermediate precision). As it can be observed in Table 2, the cholesterol value was 58.89 mg/100 g in day 1, 57.79 mg/100 g in day 2 and 58.29 mg/100 g in day 3, while the retinol was 7.25 µg/100 g in day 1, 7.33 µg/100 g in day 2 and 7.24 µg/100 g in day 3. RSD values for cholesterol analysis were 2.11%, 5.38 and 5.61% for the days 1, 2 and 3 respectively and the RSD values for retinol were 3.62%, 5.74% and 2.70% for the days 1, 2 and 3 respectively. The recommended RSD is preferred less than 1%, being values up to 10%

298 generally acceptable (CDER 1994; AOAC 2002). Repeatability RSD (for eight replicates
299 on day 1) of cholesterol and retinol analysis was 2.11% and 3.62%, respectively. These
300 values are lower than the maximum acceptable (10%) recommended by guidelines for
301 method validation. Intermediate precision was obtained after saponification, extraction
302 and injection of eight replicates, during three days. The mean of cholesterol amount of
303 the 24 replicates was 58.32 mg/100 g (Min = 53.36 and Max = 63.32 mg/100 g) and
304 retinol amount was 7.27 µg/100 g (Min = 6.65 and Max = 7.84 µg/100 g). RSD values of
305 4.49% for cholesterol and 3.99% for retinol were obtained. As occurs in the repeatability,
306 the values of RSD obtained are lower than the maximum acceptable (10%). In addition,
307 the ANOVA test for 24 replicates in the 3 days did not show any significantly differences
308 ($P = 0.72$ for cholesterol and $P = 0.83$ for retinol) between the different days of analysis.
309 Moreover, the reproducibility RSD (for 24 replicates on 3 days) of 4.49% for cholesterol
310 and 3.99% for retinol were similar numerical ranges as those of intralaboratory
311 repeatability. According to these results, the present method showed high repeatability
312 and reproducibility.

313 **Conclusions**

314 A normal-phase HPLC method for identification and quantification of cholesterol and
315 retinol from meat (beef) was developed and validated in this study. This method extracted
316 both compounds from meat using a direct saponification, without need for lipid
317 extraction, evaporation, reconstitution and derivatization needed, as compared other
318 methods. Thus, the present method allow us quantified cholesterol and retinol very fast
319 (55 minutes for saponification and extraction process and 15 minutes of HPLC analysis)
320 and using little amount of organic solvent for each sample (2.75 mL of ethanol, 0.3 mL
321 of 2-propanol and 17.7 mL of n-hexane including saponification, extraction process and

HPLC analysis). The results from this study were comparable to most recent studies in precision and accuracy, which were shown to be quantitatively applicable. As a general conclusion, the present method showed sensitive with very low LOD and LOQ for both compounds, high recoveries (mean of 100.4% for cholesterol and 95% for retinol) and high repeatability and reproducibility. Moreover, no effect of the samples matrix in chromatographic analysis was observed, and the peaks are well separated in a very clean chromatogram. Finally, the present method could be used not only in fresh meat samples but also variety of meat products. However, further validation is recommended and can be useful for an application of this modified method in other types of meat samples.

Compliance with Ethics Requirements

The authors declare no conflict of interest in this article. This article does not contain any studies with human or animal subjects.

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400

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Figure captions

Figure 1. Graphical representation (three replicates) of the calibration curves of cholesterol (a) and retinol (b) used in this study.

Figure 2. Example of HPLC chromatograms of cholesterol and retinol. (a) Cholesterol in standard solution; (b) cholesterol in meat sample; (c) retinol in standard solution; (d) retinol in meat sample.

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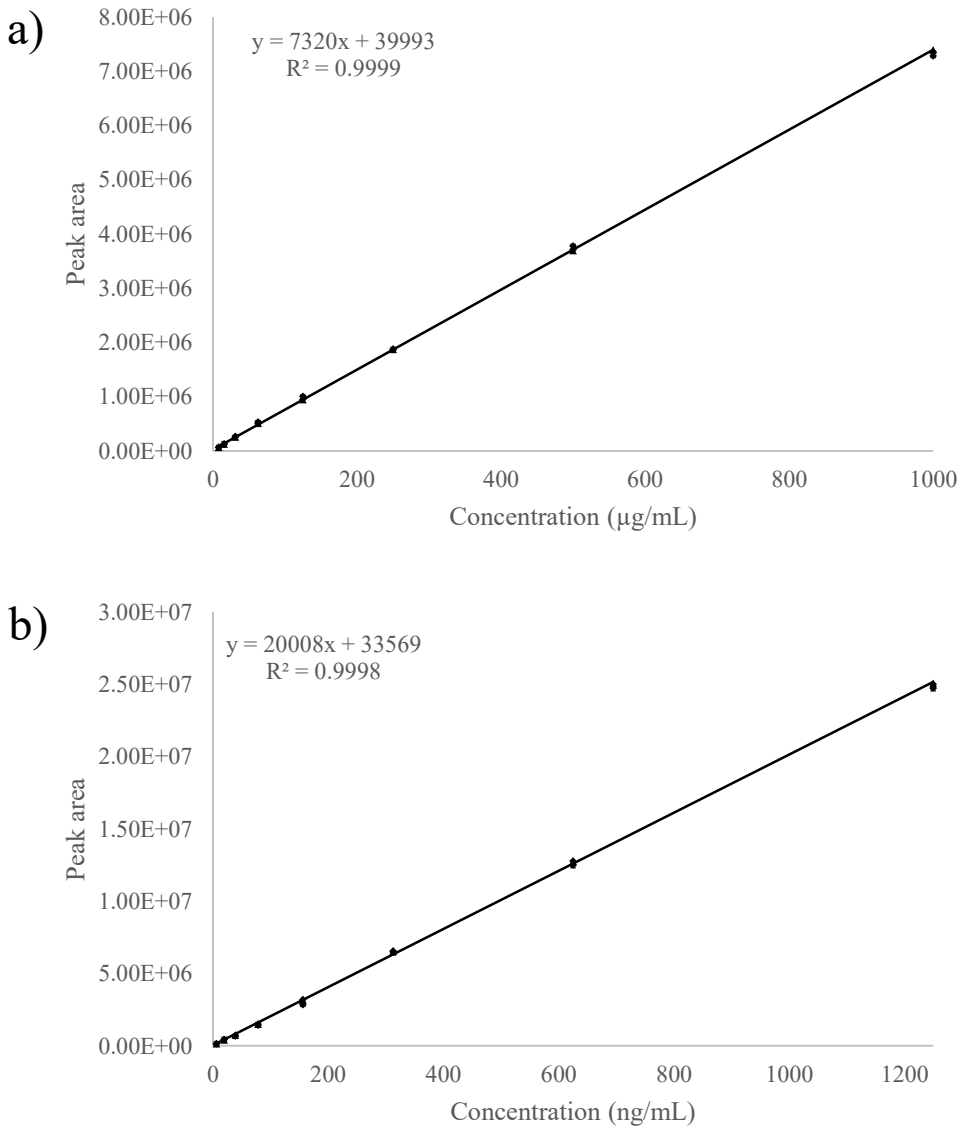

Fig 1.

Figure

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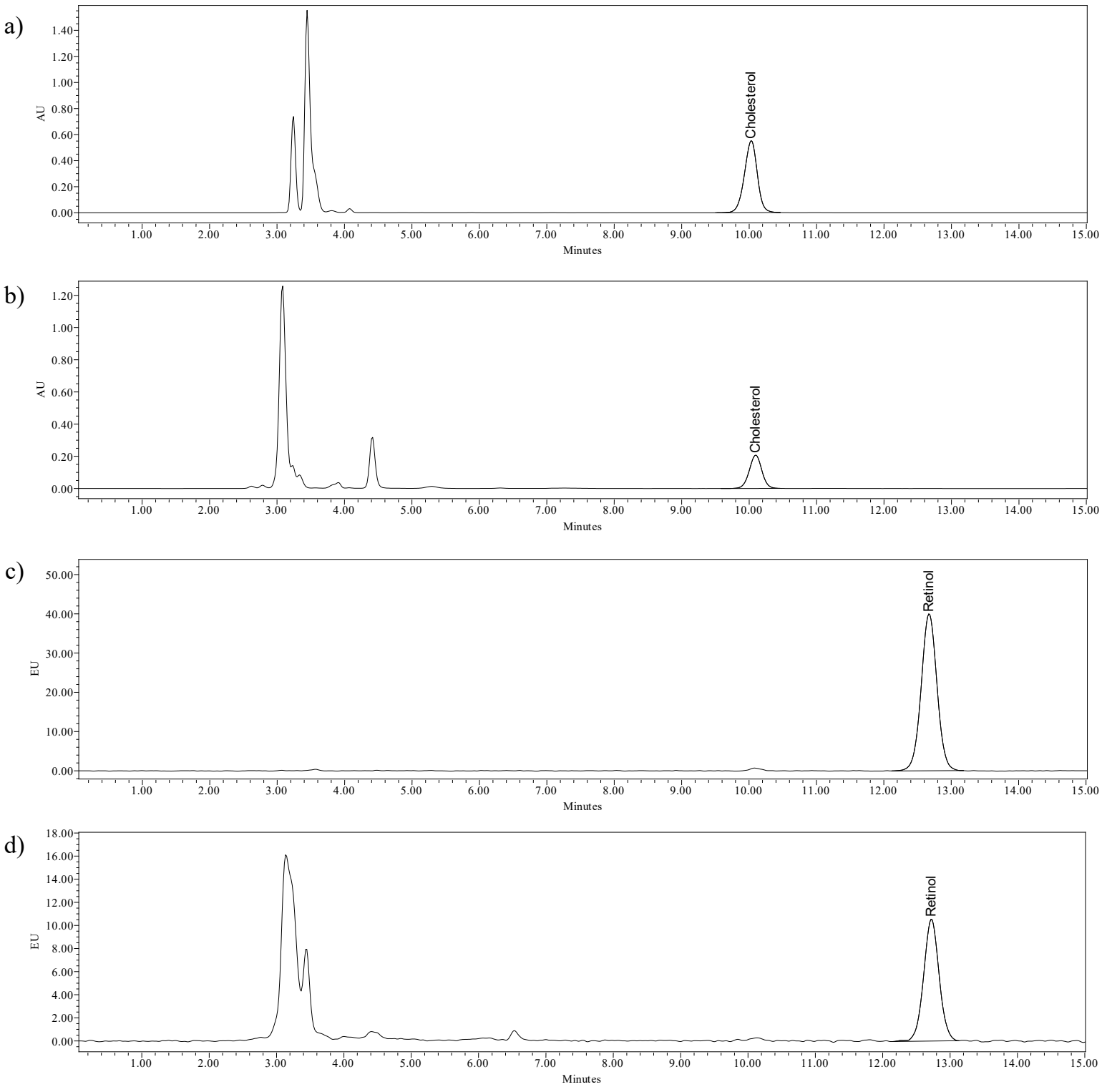

Fig 2.

Table Click here to download Table Table_1.docx

Table 1. Analytical characteristics of the developed HPLC-PDA-FL method for the simultaneous determination of cholesterol and retinol in meat.

	Cholesterol	***Retinol***
Linearity [1]		
Calibration range	8-1000 µg/mL	20-1250 ng/mL
Response factor (Area/amount) (n=6)	8.11±0.72 A/(µg ·L^{-1})	19.99±1.60 A/(ng·L^{-1})
Slope (n=6)	(7.32±0.04) x 10^3	(2.00±0.01) x 10^4
Intercept (n=6)	(4.00±1.37) x 10^4	(3.36±0.55) x 10^4
R^2 (n=6)	0.9996-0.9999	0.9996-0.9998
Baseline noise (AU) (n=10)	0.0017±0.0002	0.45±0.03
LOD [2]	3.90 µg/mL (0.59 mg/100 g)	6.25 ng/mL (0.94 µg/100 g)
LQD [3]	7.80 µg/mL (1.17 mg/100 g)	19.55 ng/mL (2.93 µg/100 g)
Peak characteristics		
Retention time (min) (n=72) [4]	10.00±0.008	12.66±0.014
Peak width (min) (n=48) [5]	0.856±0.073	0.782±0.052
Specificity (peak resolution) (n=24) [6]	15.53±2.103	14.41±0.648
Peak asymmetry (tailing factor) (n=72) [7]	0.19±0.040	0.14±0.027
Accuracy (Recovery %)		
Cholesterol (95 µg/g); Retinol (45 ng/g) (n=6)	98.95±6.41	96.60±7.09
Cholesterol (190 µg/g); Retinol (105 ng/g) (n=6)	102.14±3.04	91.72±2.01
Cholesterol (300 µg/g); Retinol (240 ng/g) (n=6)	98.67±5.01	98.27±1.11

[1] Eight standard solutions at different concentration levels were injected in duplicate for 3 consecutive days

[2] LOD calculated as the concentration yielding an S/N ratio of 3

[3] LOQ calculated as the concentration yielding an S/N ratio of 10

[4] Retention time was calculated as mean of 48 standard solutions and 24 sample injections in three consecutive days

[5] Peak width was calculated as mean of 48 standard solutions injections in three consecutive days

[6] Peak resolution was calculated as mean of 24 sample injections in three consecutive days

[7] Tailing factor was calculated as mean of 48 standard solutions and 24 sample injections in three consecutive days

[8] Accuracy was evaluated as the recovery obtained for cholesterol and retinol when six different samples of meat were spiked with cholesterol and retinol standards in three different amounts.

Table Click here to download Table Table_2.docx

Table 2. Repeatability and reproducibility of cholesterol and retinol determination.

		Cholesterol (mg/100 g)	***Retinol (μg/100 g)***
Day 1 (n=8)	1	60.71	6.86
	2	58.01	7.23
	3	59.80	7.01
	4	57.74	7.43
	5	57.40	7.66
	6	59.03	7.42
	7	58.18	7.33
	8	60.23	7.07
	Mean±SD	58.89±1.24	7.25±0.26
	%RSD	2.11	3.62
Day 2 (n=8)	1	61.01	7.36
	2	55.37	6.76
	3	57.81	6.65
	4	54.05	7.46
	5	57.94	7.42
	6	55.19	7.74
	7	57.61	7.40
	8	63.32	7.84
	Mean±SD	57.79±3.11	7.33±0.42
	%RSD	5.38	5.74
Day 3 (n=8)	1	61.55	7.16
	2	61.74	7.31
	3	54.56	7.42
	4	53.36	7.05
	5	60.84	7.39
	6	57.69	7.53
	7	60.20	7.07
	8	56.40	7.01
	Mean±SD	58.29±3.27	7.24±0.19
	%RSD	5.61	2.70
Total (n=24)	Mean±SD	58.32±2.62	7.27±0.29
	%RSD	4.49	3.99
	Min	53.36	6.65
	Max	63.32	7.84
	P value	0.72	0.83