Development of a Liquid Chormatography-UltraViolet-Mass Spectrometry analytical method for malondialdehyde

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Abstract— **A method for the analysis of malondialdehyde (MDA) in food has been developed using high-performance liquid chromatography (HPLC) coupled to UV and mass spectrometry (MS) detection. Separation and detection of malondialdehyde were achieved after derivatisation of malondialdehyde with 2,4-dinitrophenylhydrazine (DNPH). Separation was achieved isocratically with 55% water/45% acetonitrile on a Polaris C18 column (150 x 2 mm, 3 µm, Varian Inc.). MDA-DNPH was detected using a PDA detector set at 307 nm and specific mass transitions in MS-MS. The developed method showed good linearity, selectivity and specificity.**

Keywords— **Malondialdehyde, liquid chromatography/UV detection/mass spectrometry.**

I. INTRODUCTION

A lot of products containing large amounts of omega-3 fatty acids can be found on the market [1,2], but unfortunately, those essential polyunsaturated fatty acids (PUFA) are known to be easily oxidized by light, temperature, etc, during food storage or processing. At the consumer level, the problem is the formation of toxic oxidation products, which may carry adverse health effects.

PUFA oxidation leads to the formation of hydroperoxydes (primary oxidation products), while the secondary degradation compounds, are mainly aldehydes. These aldehydes are relatively stable and have been shown to be cytotoxic and genotoxic by reacting with proteins and nucleic acids [3]. Malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE), acrolein and crotonaldehyde have been recently considered, in an advice of the Belgian Superior Health council, of major concern for health [4].

These aldehydes are usually detected by the TBARS (thiobarbituric acid reactive species) method [5,6,7]. This method lacks of specificity and measures the total content of aldehydes able to react with thiobarbituric acid, expressed in malondialdehyde content.

In order to better characterize the oxidation products in foods with high PUFA content, such as meat and oils, it is important to set up more specific analytical methods to assess the quantity of each of the secondary oxidation products. Therefore, in a first step, a HPLC-UV-MS method has been developed to evaluate the concentration of malondialdehyde in food samples, including oils. Separation and detection of malondialdehyde were achieved after derivatisation of malondialdehyde with 2,4-dinitrophenylhydrazine (DNPH) according to Fenaille et al. [11]. The 3 other compounds will then be added to this LC_UV_MS detection method.

II. MATERIALS AND METHODS

Chemicals : All chemicals were of analytical grade and the solvents were of HPLC grade. 3-dimethylamino-2-methyl-2-propenal (DMP), 1,1,3,3-tetraethoxypropane (TEP), trichloroacetic acid (TCA) and 2,4-dinitrophenylhydrazine (DNPH) were obtained from Sigma (St. Louis, MO, USA).

Preparation of standard curve : The MDA standard stock solution (20 mM) was prepared by acid hydrolysis of 500 µL 1,1,3,3-tetraethoxypropane (TEP) to which 5% (W/V) of filtered TCA was added to a final volume of 100 ml. Stock solution was then diluted to 200 µM with 5% (w/v) filtered TCA to be used as working solution. The external calibration consisted of 7 points: 0, 0.5, 1.0, 1.5, 2.0, 4.0 and 10.0 µM MDA corresponding to 0 to 7.2 mg/Kg of meat.

Sample preparation and MDA derivatization : 5 g of sample were weighted in a centrifugation tube,

additioned with 15 ml TCA 5% (w/v), homogenised with Turax (13000 rpm, 1 min) and centrifuged (15000 rpm, 15 min, 4°C). This extraction was repeated a second time with 10 ml TCA. Supernatants were then filtered and combined in a 50 mL volumetric flask. TCA 5% was added to a final volume of 50 mL.

Dinitrophenylhydrazone derivatives were prepared according to Fenaille et al [10]. 100 µL DNPH solution (2mM in HCl 2M) were added to 1 mL of extract (0.22 µm PTFE filter) or 1 ml of the external MDA standard, and reaction took place for 1h in the dark at room temperature. The hydrazone was extracted 4 times with 1 mL hexane. The hexane phases were combined and evaporated under a stream of nitrogen and reconstituted in 100 µl acetonitrile/water 50:50, v/v, before HPLC analysis.

HPLC-UV-MS detection.: Separation and detection of malondialdehyde as a DNPH derivative were performed using a ThermoFinnigan Spectra System P4000 HPLC system, a Spectra System UV6000LP and a ThermoFinnigan LCQ Deca ion trap mass spectrometer, equipped with an Electrospray source. Separation was achieved isocratically with 55% water/45% acetonitrile on a Polaris C18 column (150 x 2 mm, 3 µm, Varian Inc.). The solvent flow was 0.25 ml/min, column temperature was set to 40°C and UV detection was at 307 nm. Injection volume was 20 µl and samples were injected at room temperature. The mass spectrometer analysis was performed in MS/MS mode, with electrospray source in positive mode. Sheath and auxiliary gas were settled to 40 and 30 respectively. Spray voltage was set at 6.5 kV, capillary temperature and voltage were set at 275 °C and 32 V respectively. Isotopic dilution technique was used for quantification using methyl-malondialdehyde as internal standard and quantitative results were calculated using XCalibur software. Malondialdehyde was detected following three specific fragmentations (235>189, 239>159 and 235>205).

III. RESULTS AND DISCUSSION

Selectivity/Specificity

The developed method shows good selectivity and specificity as shown on Figures 1 and 2.

Figure 1 shows the chromatographic separation and detection in UV and MS-MS of MDA-DNPH at 0 µM (blank) and 0.5 µM MDA. In the blank solution, peak of MDA-DNPH is absent as seen in Fig. 1A (MS-MS) and Fig. 1B (UV). Figures 1C and 1D show the peak of MDA-DNPH eluting between 4 and 5 minutes. These peaks correspond to a concentration of 0.5µM in the calibration curve. An interfering peak can be seen at approximately 4 min on Fig. 1B and 1D. This is likely to be a by-product of the derivatisation step.

Figures 2A and 2B show the endogenous presence of MDA in a meat sample, detected in MS-MS and UV respectively.

Linearity

Linearity was checked in a first step without using any internal standard. It was estimated as good for UV and MS-MS detection techniques from 0.5 µM to 10 µM.

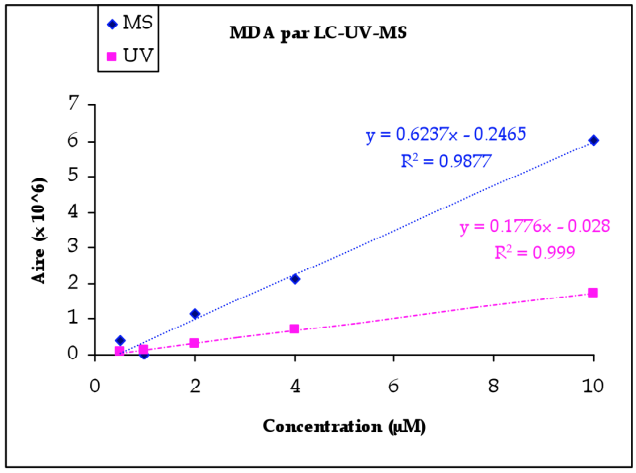

Figure 3: Calibration curves of MDA-DNPH in MS-MS and UV.

Figure 3 shows the calibration curves established in the two detection modes and the equation of each curve and the corresponding R^2. R^2 was found higher than 0.99 in both curves.

We can note however a better sensitivity of the MS calibration curve, which shows a higher slope than the UV one.

Internal Standard

The synthesis of a sodium salt of methyl-malondialdehyde (Me-MDA) is being set up in order to use it as internal standard (IS), according to literature [8,9,10].

IV. CONCLUSIONS

A specific, selective HPLC-UV-MS-MS method has been developed to analyse MDA, as a DNPH derivative. The calibration curves established in a fist step without internal standard showed good linearity, with R^2 always higher than 0.99.

This method has been shown to work with meat samples, with a good specificity. The method will be adapted to analyse MDA and other toxic aldehydes in various samples, including oil, and a complete validation, according to international criteria, will be realised.

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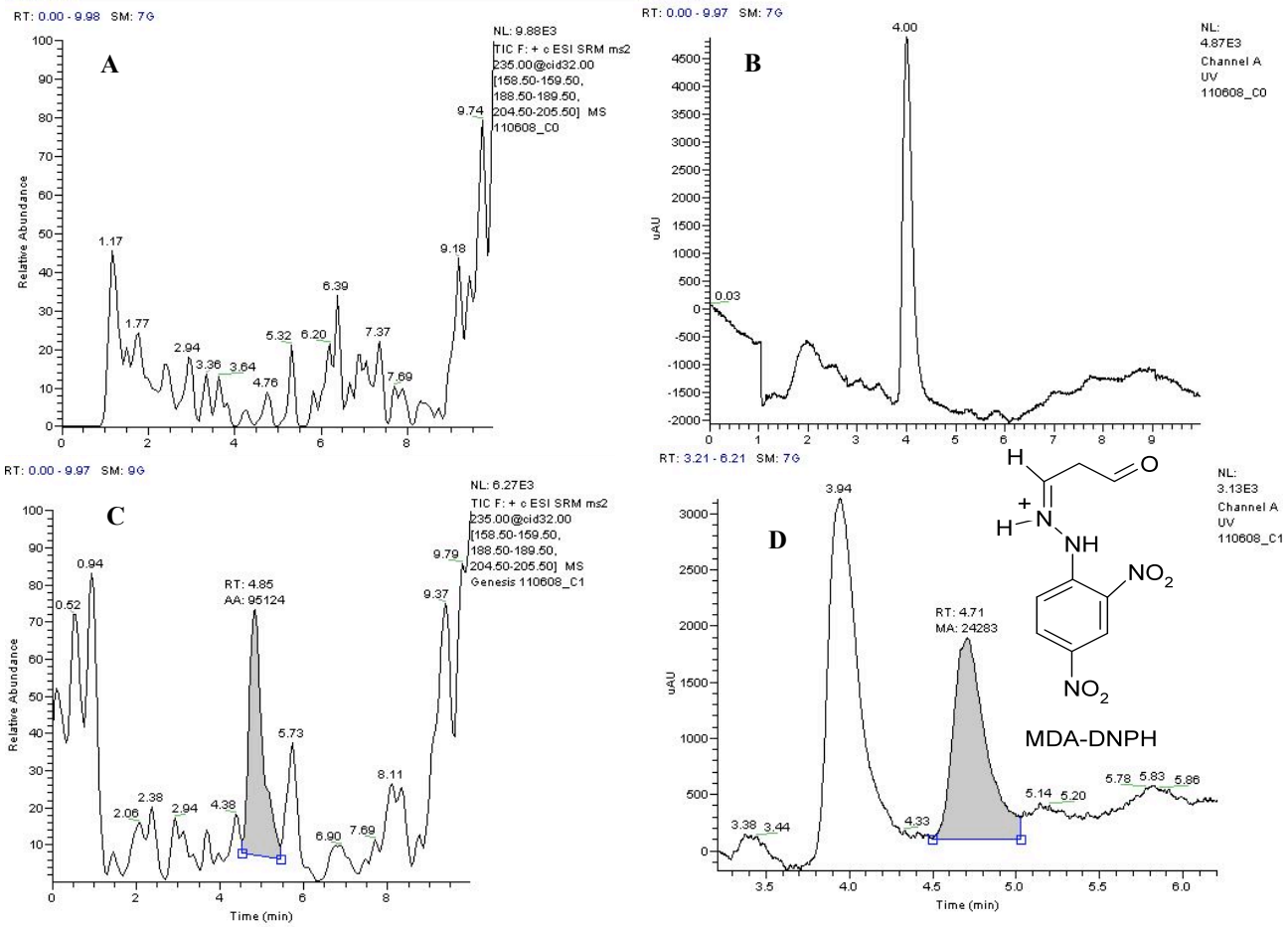

Figure 1: Chromatographic separation of MDA-DNPH. (A) and (B) correspond to a blank solution analyzed in MS-MS and in UV respectively, MDA-DNPH peak is absent. (C) and (D) show the MDA-DNPH peak, obtained from a solution with a MDA concentration of 0.5µM eluting between 4 and 5 minutes.

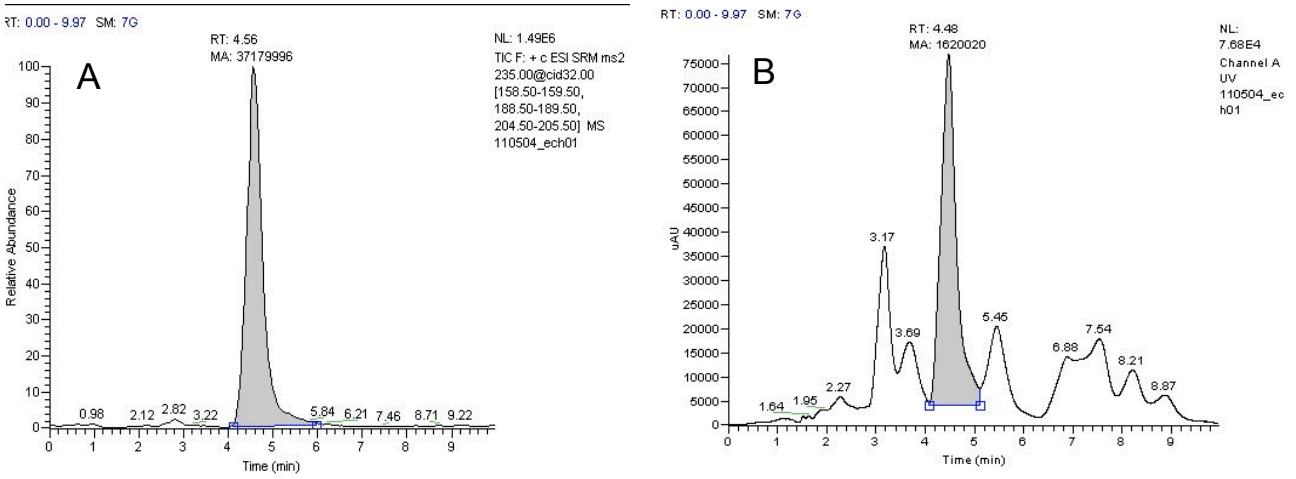

Figure 2: MDA-DNPH peak detected in a meat sample in MS-MS (A) and UV (B).