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RESEARCH PAPER

# Magnetic immunochromatographic test for histamine detection in wine

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## Abstract

Histamine, a biogenic amine, is abundant in fermented foods and beverages, notably wine. A high intake of this monoamine may produce adverse reactions in humans, which may be severe in individuals with a reduced capacity to catabolise extrinsic histamine. Thus, control of histamine concentration during wine production and before distribution is advisable. Simple, rapid, point-of-use bioanalytical platforms are needed because traditional methods for the detection and quantification of histamine are expensive and time-consuming. This work applies the lateral flow immunoassay technique to histamine detection. Superparamagnetic particle labels, and an inductive sensor designed to read the test line in the immunoassay, enable magnetic quantification of the molecule. The system is calibrated with histamine standards in the interval of interest for wine production. A commercial optical strip reader is used for comparison measurements. The lateral flow system has a limit of detection of 1.2 and 1.5 mg/L for the inductive and optical readers, respectively. The capability of the inductive system for histamine quantification is demonstrated for wine samples at different processing points (at the end of alcoholic fermentation, at the end of malolactic fermentation, in freshly bottled wine, and in reserve wine). The results are validated by ultra-high-performance liquid chromatography.

**Keywords** Biogenic amines · Histamine · Lateral flow immunoassay · Superparamagnetic nanoparticles · Histamine biosensor

## Introduction

Biogenic amines (BAs) are basic nitrogenous compounds with low molecular weight and biological activity. The most frequent among them are histamine, tyramine, tryptamine, putrescine and cadaverine, and are present in several foodstuffs in a variable range of concentrations depending on the food [1]. BAs are produced by certain bacteria and yeasts during the fermentation of wines, and other beverages and foods as beer, chocolate or cheese. They can also be produced by the normal metabolic activity of animal and vegetal cells. However, in fermented products, where BAs can reach the highest concentrations, they are normally generated by the microbial decarboxylation of the corresponding amino acids. In some cases, these microorganisms, Gram-positive and Gram-negative bacteria, are part of the starter and/or the secondary microbiota, necessary to produce the desired fermentation of the product. In other cases, they are present as food contaminants [2].

Histamine is one of the most abundant and toxic BAs in fermented foods. Even in small amounts it can produce symptoms in susceptible individuals, and in high levels it can cause serious toxicological problems [3]. In alcoholic beverages, the toxic effects can be stronger due to the inhibition effect of alcohol on the intestinal epithelium detoxification system [4], which is one of the main reasons why it is so important to analyse wines.

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Despite the risk of histamine, there is no consensus in the legislation to regulate its concentration in food. Only in fishery products the maximum histamine levels have been set at 50 mg/kg by the US Food and Drug Administration [5] and at 100 mg/kg by the European Community [6]. In the case of wine, there are no legal restrictions, but some European countries recommend upper limits that range from 2 to 10 mg/L depending on the country [7–9]. These considerations need to be taken into account to facilitate commercial transactions.

But, besides the health risks of BAs, they can also affect the organoleptic quality of the wine. It has been reported that histamine can be generated during different stages of the winemaking process: alcoholic fermentation by yeasts [10, 11], malolactic fermentation by bacteria [12, 13] and ageing [14]. Other factors such as time, storage conditions (temperature and pH), raw material quality and possibility of contamination during ageing can contribute to increase histamine content. All kind of wines may contain BAs, but typically red wines have more than other varieties (white, rose, rice, and Porto) due to their vinification processes [15], and even high-rated wines may have them.

These considerations have prompted attentive wine producers and consumers to get interested in bioanalytical methods that are fast and unsophisticated to detect and quantify BAs for sensitive individuals and wine producers both during the winery process and in the final product.

Up to now, different methods have been reported to detect BAs. Currently, the most used analytical technique is liquid chromatography (LC) coupled to ultraviolet, fluorescence or mass spectrophotometry detectors [16]. Gas chromatography (GC) is a faster alternative for which pre-derivatisation is required to increase the volatility and decrease polarities of the BAs [17]. Capillary electrophoresis (CE) is the second most common technique for BA determination because it is very adequate for screenings [18]. The chromatographic techniques described above (LC, GC and CE) give a precise and sensitive analysis of numerous BAs simultaneously. However, they are time-consuming and require expensive instruments and qualified personnel. Non-chromatographic analyses are alternatives that include biosensors [19–21], enzyme-linked immunosorbent assay (ELISA) [22] and flow injection analysis [23]. These methods are faster but require sample pretreatment to clean up matrix interferences. Moreover, they still require specialised personnel and are difficult to implement in a cellar, where a rapid respond with the minimum complexity is needed.

Lateral flow immunoassay (LFIA) is a powerful point-of-use system for simple, rapid, portable and low-cost analysis in different fields (clinical diagnosis, food safety and environment). The LFIA technique is based on an immunochromatographic separation at nitrocellulose dipsticks, as sample flows by capillary action. The test involves different cellulosic materials (sample pad, conjugate pad, nitrocellulose membrane and adsorption pad) assembled on a plastic backing to get robustness. The recognition of the analyte relies on the use of specific molecules immobilised on the membrane in two lines (test and control lines). LFIA combines the technology of ELISA and chromatography, overcoming some drawbacks of both methods, such as time-consumption and complexity. There are different formats depending on the analyte. The most common are sandwich format, in which two antibodies are used to recognise the analyte, and competitive format for low molecular weight compounds, such as histamine, in which a single antibody is available for the recognition of the molecule of interest. Several systems have been used as reporter labels in LFIAs: gold, iron oxide, carbon, selenium or silver nanoparticles (NPs), coloured latex beads, quantum dots, enzymes or liposomes. Frequently, LFIA operates on a purely qualitative basis, displaying a positive/negative answer. Motivated by the concern on fishery products, lateral flow devices for a rapid visual detection of histamine have been developed [24], some of which can be applied also to wine. These tests are aimed at detecting unsafe levels of histamine according to legal regulation on fish, so they provide a positive/negative response with a threshold of 50 ppm or 200 ppm.

This article focuses on adding quantitative capacities to LFIAs for histamine in red wine, while reducing the LOD to levels to match the European recommendations for this product (2–10 ppm). Associating the LFIA to a reading equipment which does not compromise the rapidity and simplicity of the test is a major challenge [25]. Recently, magnetic nanoparticles have been proposed as LFIA labels to enable, besides the visual detection, magnetic quantification [26–29]. The authors have reported on a novel inductive sensor to quantify superparamagnetic particles without the application of exciting fields, which largely reduces the complexity and cost of the device [30, 31]. The system, adapted for LFIA strips, has been successfully used to determine prostate-specific antigen concentrations in the clinical range of interest by using a sandwich format [32]. This measuring device does not require bulky components. This means that it can be easily miniaturised to a point-of-use portable device.

In this article, we use this novel approach to detect and quantify histamine, in the concentration range of interest for wines. Although the thresholds already adopted as recommendation in some EU countries are 2–10 mg/L, studies on 100 selected high-quality red wines made from seven different cultivars found that 34% were above this limit and as high as 27 mg/L [9]. The range of interest widens then to 1–100 mg/L.

We report for the first time on a histamine quantification technique for red wine based on a magnetic competitive LFIA. Red wine is especially challenging to its intensely coloured matrix that implies high background and poor reliability for

the traditional optical readers. The purple colour of red wine aggravates the problem as the classical labels are gold NPs that yield a similar red-purple colour. Therefore, this work was aimed at developing a LFIA based on superparamagnetic labels. The immunoassay is combined to an inductive sensor capable to quantify the magnetic moment of such labels. The system has been tested with red wine samples and validated against ultra-high-performance liquid chromatography (UHPLC) analyses. The system proved to be successful as a histamine point-of-use analytical technique in the range of regulatory concern.

## Materials and methods

### Reagents and instruments for the immunoassay

Mouse histamine monoclonal antibody (MBS2025715) and histamine-BSA conjugate antigen (MBS358205) were purchased from Mybiosource. Anti-mouse IgG, bovine serum albumin (BSA), 1-ethyl-3-[3-dimethylaminopropyl]-carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), 2-(*N*-morpholino)ethanesulfonic acid (MES) and histamine dihydrochloride were provided by Sigma-Aldrich (Spain). Recombinant protein A/G was purchased at Thermo Scientific (MA, USA). Gold nanoparticles of size 40 nm were purchased from BB International (UK). Disposable 0.45-μm PVDF filters were purchased from GE Healthcare Life Sciences.

Glass fibre membrane (GFCP001000) used as sample pad and backing cards (HF000MC100) were purchased from Millipore (Germany). Other materials used were nitrocellulose membranes (UniSart CN95, Sartorius, Spain) and absorbent pads (Whatman, USA). Based on previous results, the sample buffer consisted of 10 mM phosphate buffer (PB) pH 7.4 with 0.5% Tween-20 and 1% BSA.

An IsoFlow reagent dispensing system (Imagene Technology, USA) was used to dispense the detection lines (dispense rate 0.100 μL/mm) and the strips were cut with a guillotine Fellowes Gamma (Spain). A portable strip reader ESE Quant LR3 lateral flow system (Qiagen Inc., Germany) was used to quantify the intensity of the test line by reflectance measurements.

### Functionalisation of labels with protein A/G

Protein A/G was conjugated to gold nanoparticles for its functionalisation. A gold colloidal titration was carried out to find the optimal concentration of protein A/G to stabilise the gold nanoparticles. The titration experiments show that 1.5 mg/mL of protein is the optimal concentration for its functionalisation. For the conjugation, 100 μL of 1.5 mg/mL protein A/G was added to 1.5 mL of gold nanoparticles in suspension. After shaking for 1 h, 100 μL of blocking solution (1 mg/mL BSA in PB 10 mM, pH 7.4) was added to block the residual surfaces of the gold conjugate. After 20 min of blocking reaction, the mixture was centrifuged at 6800*g* for 20 min. The supernatant was discarded, and the pellet was resuspended in 2 mM of PB buffer, pH 7.4, with 10% sucrose and 1% BSA. The conjugate protein A/G-gold nanoparticles were stored at 4 °C until used.

Superparamagnetic magnetite nanoparticles prepared by a coprecipitation route and coated with a double layer of oleic acid [33] were functionalised using recombinant protein A/G to develop the immunoassay. Firstly, the carboxyl groups of the nanoparticles were activated using the carbodiimide chemistry. For this, 100 μL of EDC (5 mg/mL in MES 50 mM, pH 6.00), 100 μL of NHS (5 mg/mL in MES 50 mM, pH 6.00) and 100 μL of recombinant protein A/G (different concentrations were used to optimise the assay) were mixed with 10 μL of nanoparticles. After shaking for 4 h, the residual carboxyl groups on the surfaces were blocked by adding 100 μL of the blocking solution (1% BSA in PB 10 mM, pH 7.4). Then, the mixture was centrifuged at 21,448*g* for 20 min. Finally, 300 μL of supernatant was discarded and the pellet was resuspended in PB 10 mM, pH 7.4.

### Characterisation of nanoparticle conjugates by dynamic light scattering

Size distribution and ζ potential assays were carried out with a Zetasizer Nano ZS ZEN3600 (Malvern Instruments, Malvern, UK) equipped with a solid-state He–Ne laser (633 nm). This instrument was used to monitor the conjugation process. A total of three readings were carried out at 25 °C. Each reading was composed of 15 measurements of the backscattered (173°) intensity. Zetasizer software version 7.03 was used for data processing and analysis.

### Preparation of the immunostrips

The competitive LFIA to detect histamine was carried out in a dipstick format. The nitrocellulose membrane (25 mm wide) was incorporated into a backing plastic card to make it robust enough. Two lines of antibodies were immobilised across the nitrocellulose strip: (i) the test line to provide the result of the analysis and (ii) the control line to get the guarantee that the liquid sample has flowed adequately along the strip. Both lines were applied by the IsoFlow dispenser at a rate of 0.100 μL/mm. A 1 mg/mL concentration of histamine-BSA conjugate was used for the test line and a 1 mg/mL concentration of anti-IgG for the control line. The nitrocellulose membrane was dried for 20 min at 37 °C after the immobilisation of the control and test lines. Then, the sample pad and the absorbent pad were settled onto a backing card with an

overlap between them of 2 mm. The complete strip was cut into individual 5-mm-wide dipsticks.

### Magnetic quantification

To provide a quantitative signal of the test line, an inductive sensor specially developed for strip immunoassays was used [32]. Its sensing head consists of a double copper line printed on a rigid insulating substrate across which alternating current flows. The magnitude and phase of the sensing head impedance are continuously monitored by a precision impedance analyser (Agilent 4294A) using 16048G test leads and 500-mV/20-MHz excitation voltage. The change of magnetic permeability produced by the particles in the surrounding of the conductor increases significantly its impedance. The test lines on the strips were scanned laterally over the sensing head by a micro-positioner, producing a peak of the impedance signal over the base line that is integrated to account for all the particles in the line no matter how they are distributed. The signal provided by the sensor is then measured in Ω•mm coming from the cumulative integral of the impedance (Ω) across the width of the test line (mm). This alteration in the impedance is directly proportional to the number of superparamagnetic nanoparticles at the test line. As the magnetic NPs were used in the strips as labels, this approach was used to calibrate the quantification of histamine. After the calibration with histamine standards, a similar procedure was used to quantify the concentration of histamine in real red wine samples.

### Optical measurements

A portable strip reader ESE-Quant LR3 lateral flow system (Qiagen Inc., Germany) was used to quantify the colour intensity of the test line by reflectance measurements. The optical reader analyses the reflectance at the control and test line by using two channels (LED excitation and photodiode detection). The reader scans the strip by illuminating it with a light beam and then measures the attenuation from the surface of the strip through a confocal detector. The detector registers the signal and converts it into an electrical signal that is related to the amount of analyte at the control and test lines. The device provides values in units of mm mV, resulting from integrating the electrical signal (mV) across the width of the test line (mm).

### Validation by ultra-high-performance liquid chromatography measurements

The wine samples were prepared and analysed by UHPLC following a method reported elsewhere [34]. The samples analysed correspond to red wine from different elaboration stages, at the end of the alcoholic fermentation (sample A), freshly bottled (sample B), reserve wine (sample C) and at the end of the malolactic fermentation (samples D and E), all from the Vino de Cangas Protected Origin Region (from Asturias in Spain).

## Results and discussion

### Optimisation of the immunoassay

The first step to develop the immunoassay was to optimise the amount of protein used to coat the nanoparticles and the concentration of antibody.

#### Protein A/G concentration for nanoparticle conjugation

Different concentrations of recombinant protein A/G were tested (1, 2, and 3 mg/mL). Dynamic light scattering (DLS) measurements were carried out to confirm the conjugation reaction. This technique allows comparison between nanoparticle hydrodynamic sizes before and after the conjugation reaction. The hydrodynamic diameter of nanoparticles before conjugation was 86.6 nm (PDI 0.2). The ζ average of the hydrodynamic sizes was 479.8 nm (PDI 0.4), 193.0 nm (PDI 0.4) and 120.8 nm (PDI 0.3) after conjugation with 1 mg/mL, 2 mg/mL and 3 mg/mL of protein, respectively (Fig. 1). The results showed that, for all concentrations of protein A/G, the nanoparticle size increased after the addition of the protein. This demonstrates that the conjugation process through the carbodiimide chemistry was successful. Furthermore, the diameter of the conjugates was larger when concentration of the protein was lower. This could be

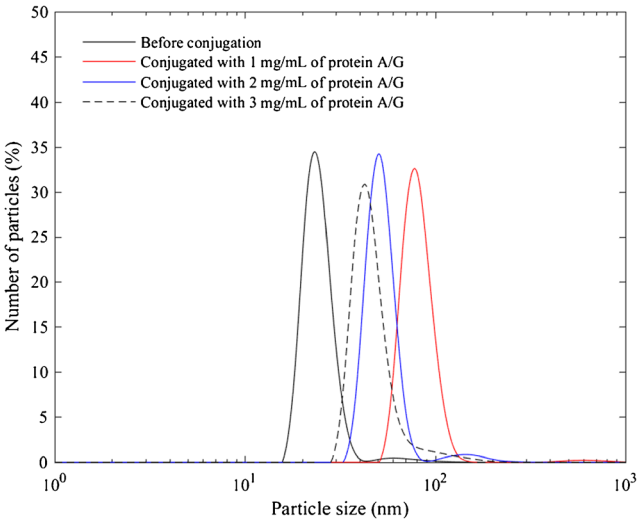



**Fig. 1** Hydrodynamic size distribution profiles of superparamagnetic iron oxide nanoparticles before (solid black line) and after conjugation with concentrations of 1 mg/mL (red), 2 mg/mL (blue) and 3 mg/mL (dashed) of protein A/G

explained because a protein molecule could bind to several nanoparticles when there were not enough molecules to cover every single nanoparticle; thus, aggregates were formed.

With the aim to select the most suitable protein concentration, the immunoassay without histamine (blank sample) was carried out in the range 1–3 mg/mL and the line tests were analysed by reflectance measurements using reader ESE-Quant LR3 lateral flow system. In all cases, the aggregates were able to flow through the nitrocellulose membrane by capillarity. The results of the measurements of the test line yielded values of 909.99, 845.73 and 639.40 mm•mV for 1 mg/mL, 2 mg/mL and 3 mg/mL of protein, respectively. The reflectance signal increases when the diameter of protein A/G-NP conjugates is larger because in this case, there are more NPs attached to each antibody unit. These agglomerates have a signal amplification effect, proportional to the size of the conjugate. In view of these results, we concluded that 1 mg/mL of recombinant protein A/G produced the largest signal. Therefore, this was the concentration chosen for the next experiments.

### Anti-histamine antibody concentration

Different concentrations of antibody were assayed in order to optimise the signal. This step was made with gold conjugates and used to estimate the antibody concentration for the competitive assay. The antibody was added during the first step of the immunoassay and therefore, direct binding to the histamine-BSA complex immobilised on the membrane occurs. For the immunoassays, 13, 10, 9 and 5 mg/L of antibody were used. The reflectance measurements yielded 996.97, 984.62, 862.05 and 431.48 mm•mV, respectively. Finally, 10 mg/L was chosen because, even when the signal increases with the concentration, there were no significant differences between 10 and 13 mg/L.

## Competitive lateral flow immunoassay procedure

The test to quantify histamine is based on a competitive immunoassay; thus, the relationship between the concentration and signals, either magnetic or optical, tends asymptotically to zero. The procedure consists of two steps, as illustrated in Fig. 2. The first step is the competition of the anti-histamine antibodies in solution for the histamine in the sample and the one immobilised at the test line. The second step is the colour developing, based on the retention of the conjugate protein A/G-NP at the test line thanks to the ability of protein A/G to bind to antibodies of any kind. The surplus of protein A/G-NP (not retained at the test line) proceed along the strip and are trapped at the control line by the Fc region of the anti-IgG and anti-histamine antibodies (see Fig. 2).

In order to calibrate the strips, several histamine standard solutions were prepared in 10 mM PB and pH 7.4. The competitive LFIA was carried out in dipstick format. For the first step, 10 μL of histamine of different concentrations and 2 μL of anti-histamine antibody (0.5 mg/mL) were transferred into a microtube from a stock solution to get a final concentration of the anti-histamine antibody of 10 mg/L. Then buffer was added, always keeping a final volume of 100 μL (98 μL of buffer for the blank and 88 μL of buffer for the samples). The optimised running buffer had 1% BSA. The sample pad was introduced into the mixture and the solution flowed along the strip by capillary action. After 30 min, 10 μL of NPs coated with protein A/G and 90 μL of running buffer were added to the microtube for the developing step.

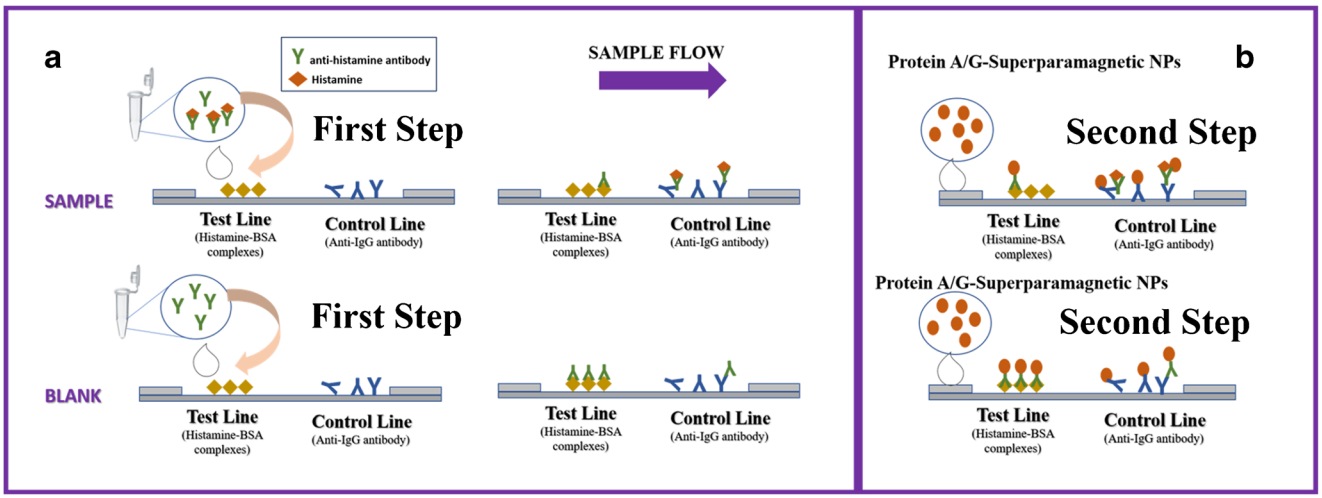



**Fig. 2** Schematic illustration of the competitive LFIA. **a** First step: immobilisation of the anti-histamine antibodies. **b** Second step: colour development

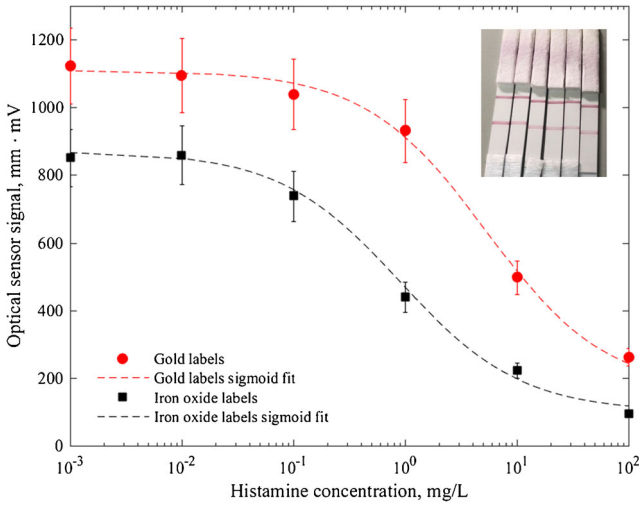



**Fig. 3** Quantification of LFIAs by the optical reader using gold (red) and iron oxide (black) NPs. The lines plotted to guide the eye are four-parameter sigmoid curves. Inset: One of the series of standard samples used to obtain the red curve, with the control line on top and the test line below

## Histamine standards calibration

To calibrate the strips, several histamine standard solutions were prepared by dilution and assayed following the procedure previously described. In a first run, the experiments were conducted in a wide range of histamine concentrations. The protein concentration in the functionalisation protocol was 1.5 and 2 mg/mL for gold and magnetic nanoparticles respectively. Figure 3 shows the calibration curves in the immunoassays using gold (red) and iron oxide (black) NPs as labels. Six standard samples with histamine concentrations in the range from $10^{-3}$ to $10^{2}$ mg/L, besides a negative reference sample, were run by triplicate and measured with the optical reader. The results yield sigmoid-shape profiles, characteristic of competitive immunoassays. For very low concentrations, the curve has an asymptotic behaviour as a result of the total occupancy of the immobilised histamine-BSA complexes by anti-histamine antibodies. The measured points have been fitted in Fig. 3 (dotted lines) using the four-parameter logistic equation:

$$S = \frac{\alpha - \delta}{1 + (C/\gamma)^{\beta}} + \delta \qquad (1)$$

where $S$ and $C$ are the sensor measurement and the histamine concentration, $\alpha$ and $\delta$ are the $S$-values of the upper and lower asymptote, respectively, $\beta$ is the slope at the inflexion point, and $\gamma$ is the value of $C$ corresponding to 50% of the maximum asymptote [35]. The parameter values used for the fits of Fig. 3 were respectively $\alpha = 1110$ mm•mV, $\beta = 0.8$, $\gamma = 5.3$ mg/L, and $\delta = 160$ mm•mV (red line) and $\alpha = 1130$ mm•mV, $\beta = 0.8$, $\gamma = 4.5$ mg/L, and $\delta = 190$ mm•mV (black line). This first analysis leads to the conclusion that quantification is feasible in the range of 1–100 mg/L for the gold nanoparticles and 0.1–100 mg/L for the iron oxide superparamagnetic particles. Besides their wider working range, iron oxide NPs have the advantage of enabling the magnetic pre-concentration of the analyte. Although this procedure has not been yet tested in histamine immunoassays, it has been commonly used for pre-concentration of analytes associated to different bioanalytical techniques [36]. This allows bringing the samples with lower concentrations into the working range by a simple magnetic pre-treatment.

Figure 4 shows a detailed calibration with magnetic NPs in the range 1–100 mg/L. For this, the A/G protein concentration

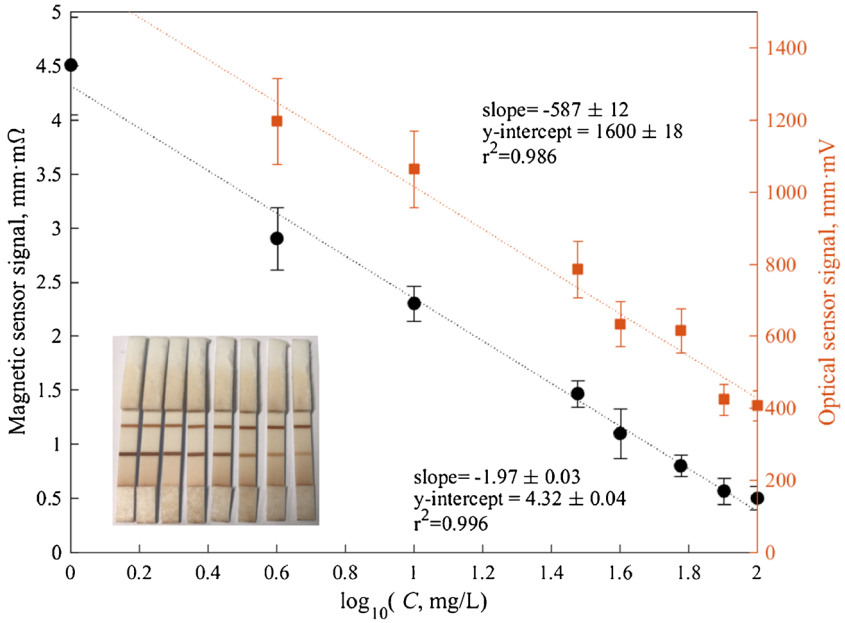



**Fig. 4** Inductive (left axis) and optical (right axis) signals of the magnetic LFIA as a function of the concentration of histamine. Least-squares linear regression curves are plotted as dotted lines. Inset: One of the series of standard samples used to obtain the plotted lines. The control line is on top and the test line below

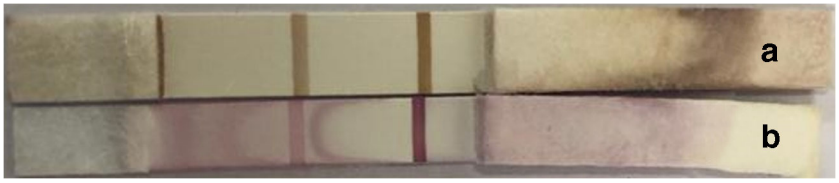



**Fig. 5** LFIAs run with red wine samples using (**a**) superparamagnetic nanoparticles and (**b**) colloidal gold as reporters

for functionalisation was optimised according to the DLS results. Optical and inductive readers have been used, the latter yielding better correlation factor. Dotted lines represent the least-squares linear fit of the data; slopes, *y*-intercepts and correlation factors are also shown on the graph. The limits of detection (LOD) have been calculated following ref. [37] from the blank-subtracted results and give values of 1.2 mg/L and 1.5 mg/L for the inductive and optical methods, respectively.

## Application to wine samples

The analytical method was tested against five samples of red wine taken at different stages of the fabrication. The only pretreatment was a simple filtering through a 0.45-μm PVDF filter. All the assays were performed in triplicate. The strips were measured with the inductive reader once dried, to get a quantitative reproducible reading.

Initial tests were done with both types of particles, iron oxide and the traditional colloidal gold. For comparison, Fig. 5 shows an image of both types of strips after running a sample of red wine. Even a naked eye inspection allows concluding that red wine stains the white paper. This is especially remarkable in the case of gold labels which seem not to flow as well as magnetite. In such case, a quantitative determination by optical measurements becomes impossible or is very poor. For this reason, together with the better results yielded by the calibration, the combination of iron oxide NPs and inductive sensor was chosen to perform the histamine quantification. The results are summarised in Table 1.

To validate the results, wines were also analysed by UHPLC and the results of histamine content are compared in Table 1. The error in the UHPLC measurements comes from the injection volume, which has been considered the only significant source of error. In the case of the LFIA, several error sources were identified that influence the inductive signal value: the printing of the antibody across the membrane (which may have small inhomogeneities in the line width), the guillotine cut of the strip from the membrane original card (this produces variability in the width of the strips), and the sensor resolution. Considering that the errors produced are probabilistically independent, we can use the rule of error propagation to add up the relative errors. For this calculation, three strips have been run with each wine and each of them has been measured four times. The resulting uncertainty of the sensor signals for the different wines is in the range 2–18% for the optical measurement and 2–5% for the inductive sensor. The uncertainties of the concentrations shown in Table 1 have been obtained by the propagation rule applied to the calibration curve considering the errors of the slope and *y*-intercept given in Fig. 4.

The LFIA values of the histamine content measured with the commercial optical reader are deficient. Moreover, the relative uncertainties are unacceptable due mainly to a considerable variability in the three strips of each wine. The problem is associated to the variability in colour intensity of the three strips of each wine more than a lack of sensitivity of the reader itself. For this method to be useful for quantification, the removal of the interfering matrix would be necessary. In contrast, the LFIA histamine levels given by the inductive reader are remarkably similar to the reference, taking into account the margins of error. Only wines D and E fall out of these ranges, the average LFIA value being overestimated in about a 30%.

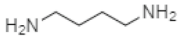
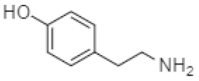
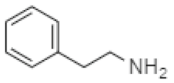
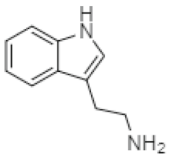
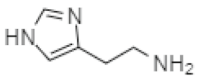
To investigate the possible origin of this deviation, several BAs have been quantified by UHPLC in the wines under study, namely, histamine, putrescine, cadaverine, tyramine, phenylethylamine, and tryptamine (see Table 2).

Of all the amines analysed, tyramine has a similar structure to histamine and reaches higher values in wines D and E, so it

**Table 1** Results obtained by magnetic LFIA coupled to the inductive sensor and UHPLC for the red wine samples analysed in this work

| Wine | Stage of fabrication | LFIA and inductive sensor mg/L\|mM | | LFIA and optical sensor mg/L\|mM | | UHPLC mg/L\|mM | |
|---|---|---|---|---|---|---|---|
| A | End of the alcoholic fermentation | 46 ± 2 | 0.41 ± 0.02 | 25 ± 3 | 0.22 ± 0.03 | 44 ± 3 | 0.39 ± 0.03 |
| B | Freshly bottled | 82 ± 4 | 0.74 ± 0.04 | 56 ± 14 | 0.50 ± 0.13 | 83 ± 3 | 0.74 ± 0.03 |
| C | Reserve wine | 25 ± 3 | 0.22 ± 0.03 | 10 ± 1 | 0.09 ± 0.01 | 20 ± 3 | 0.18 ± 0.03 |
| D | End of the malolactic fermentation | 63 ± 4 | 0.57 ± 0.04 | 37 ± 7 | 0.33 ± 0.06 | 46 ± 3 | 0.42 ± 0.03 |
| E | End of the malolactic fermentation | 65 ± 5 | 0.58 ± 0.05 | 101 ± 18 | 0.90 ± 0.16 | 55 ± 3 | 0.49 ± 0.03 |

**Table 2** Concentrations of biogenic amines by UHPLC. All the samples are red wine at different steps of the fabrication

| Biogenic amines | Structure | Wine A (mM) | Wine B (mM) | Wine C (mM) | Wine D (mM) | Wine E (mM) |
|---|---|---|---|---|---|---|
| Putrescine |  | 1.717 | 1.842 | 0.754 | 1.417 | 1.769 |
| Cadaverine |  | 0.025 | 0.050 | 0.049 | 0.063 | 0.060 |
| Tyramine |  | 0.108 | 0.375 | 0 | 0.406 | 0.623 |
| Phenylethylamine |  | 0.072 | 0.418 | 0.452 | 0.112 | 0.350 |
| Tryptamine |  | 0 | 0 | 0 | 0 | 0 |
| Histamine |  | 0.39 | 0.742 | 0.175 | 0.416 | 0.494 |

is likely to be producing a cross-reaction effect. In order to test this hypothesis, we have run the test with standard solutions of histamine (H) and tyramine (T). In one case, we have used 2.5 μg of histamine while in the other we have also added 6.5 μg of tyramine. This leads to tests with 0.22 and 0.69 mM concentration of BAs. These tests were performed with iron oxide NPs as labels and evaluated with the inductive sensor. The results are shown in Table 3 together with the "apparent histamine amount," that is obtained applying the calibration parameters given in Fig. 4. It can be concluded that the presence of tyramine interferes at the test, which displays lower magnetic signals and give rise to a value of histamine 27% larger than the nominal one. Another parameter that can be affecting these results is the different flowing velocity of the samples due to the matrix composition and viscosity.

**Table 3** Cross-reactivity study. The letters H and T stand for histamine and tyramine, respectively

| BAs | Concentration of BAs (mM) | Signal by inductive measurements (mm mV) | Apparent concentration of histamine (mg/L)/(mM) |
|---|---|---|---|
| H | 0.22 | 1.5 | 26/0.23 |
| H + T | 0.69 | 1.3 | 33/0.28 |

Although further research is needed to improve the reliability of the test, the results of LFIA are a proof of the capability of the system not only to detect, but also to quantify histamine in red wine in the range of interest for wineries and sensitive consumers.

## Conclusions

A biosensor for histamine quantification in red wines has been developed based on the combination of magnetic competitive lateral flow immunoassay strips and an inductive sensor to perform the reading out. The labels are superparamagnetic iron oxide nanoparticles 10 nm in size with a double lipidic layer as coating that enables their functionalisation. The magnetic perturbation of the NPs is detected by the inductive sensor as an increase of its impedance proportional to the number of NPs in the test line, which is in turn proportional to the number of anti-histamine antibodies. The system has been calibrated with histamine standard solutions. To validate the new method, the competitive immunoassay has been done also with traditional gold NPs and evaluated, both with gold and magnetite, with a commercial reflectance reader. The combination of magnetic particles and inductive reader gave

the best calibration correlation factor and LOD, besides the well-known magnetic pre-concentration potential.

Finally, the system was tested for reliability and validity with five real red wine samples corresponding to different stages during vinification and the final consumption state. In this case, the magnetic particles proved a new advantage compared with colloidal gold, which has a better flow of the wine sample along the paper resulting in a cleaner strip. In addition, the magnetic inductive signal does not depend on the dyeing of the paper, which is a complication for gold labels whose reading relies on an optical signal.

The measurements were cross-checked by UHPLC, leading to the finding that in two of the wine samples they can be overestimated in about 30%, probably due to cross-reactivity with tyramine. Despite this, the conclusion is that the analytical method based on magnetic LFIA is very promising for point-of-use determination of histamine. Keeping the advantages of simplicity, rapidity and low cost of traditional LFIA, the magnetic character of the labels and their detection principle provide additional advantages like the avoidance of sample pre-treatment for matrix removal, possibility of magnetic pre-concentration for low-concentration samples, and improved calibration and LOD.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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