

Highly selective solid-phase extraction sorbents for chloramphenicol determination in food and urine by ion mobility spectrometry

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Abstract Different highly selective sorbents have been evaluated for the treatment of food and biological samples to determine chloramphenicol residues by ion mobility spectrometry (IMS). Combination of a selective solid-phase extraction (SPE) and dispersive liquid-liquid microextraction allowed a highly sensitive determination of chloramphenicol in water, milk, honey, and urine samples. The performance of selective SPE supports such as immunoaffinity chromatography (IAC) and molecular imprinted polymers (MIP) have been compared in terms of selectivity, sensitivity, trueness, precision, and reusability. Quantitative recoveries were obtained for chloramphenicol residues, ranging from 91 to 123 % for water, from 99 to 120 % for skimmed milk, and from 95 to 124 % for urine using IAC-IMS and MIP-IMS methods. Quantitative recoveries (from 88 to 104 %) were also achieved for honey samples using IAC-IMS, but low recoveries were obtained using MIP-

IMS. The limit of quantification was set at $0.1 \mu\text{g L}^{-1}$ which is lower than the minimum required performance limit established by the EU. The proposed methodology is a simple and cost affordable alternative to chromatography methods for the highly sensitive and selective analysis of chloramphenicol residues in food and urine.

Keywords Chloramphenicol · Ion mobility spectrometry · Immunoaffinity chromatography · Molecular imprinted polymer · Solid-phase extraction

Introduction

Ion mobility spectrometry (IMS) has recently gained increased attention as a well-established analytical technique for the determination of volatile and semivolatile compounds in different matrices with a good sensitivity and high sample throughput. IMS is based on the gas-phase separation of the resulting ions formed under a weak electric field at ambient pressure [1]. Primarily, it was used for the analysis of explosives, illicit drugs, and chemical warfare agents in security control points. However, the analytical potential of IMS, particularly as regards operational speed and sensitivity, has extended its scope to the pharmaceutical, food and feed, clinical, polymer, petrochemical, and environmental fields [2].

IMS measurement of complex samples produces a mixture of reagent and analyte product ions [3], which could complicate the interpretation of IMS plasmagrams and interfere in analyte determination. Moreover, the measured number of theoretical plates of an IMS device operating at 3000 V, a gate width of 0.2 ms and 150 °C, rarely exceeded 5000. Compared to the typical number of theoretical plates obtained with other separation techniques, as 25,000 for liquid chromatography (LC), 120,000 for gas chromatography, and 300,000 for capillary

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electrophoresis [4], it can be easily imagine that the low separation efficiency has limited the applicability of IMS. Therefore, the incomplete resolution of peaks is a common problem in the analysis of complex samples by IMS. So, to do this kind of analysis, the use of chemometrics [5, 6] or sample pre-treatments based on diverse separation techniques such as SPE [7], solid-phase microextraction [8, 9], stir-bar sorptive extraction [10], liquid-liquid microextraction [11], single-drop microextraction [12], hollow-fiber liquid-phase microextraction [13], and paper spray [14] has been proposed. However, in most cases, the selectivity of the currently available sample treatments is not enough to remove interferences and competitive ionization problems can take place in IMS determinations.

The potential of highly selective sorbents as sample treatment in IMS has been recently demonstrated. Immunoaffinity chromatography (IAC) columns have been used for the determination of mycotoxins [15, 16] and fungicides [17] and molecularly imprinted polymers (MIPs) for the selective analysis of pesticides [18, 19], pharmaceuticals [20, 21], and nitrobenzenes [22].

The main objective of this study has been the critical comparison of the capabilities of different highly selective sorbents, such as IAC and MIPs columns, as sample pre-treatments in IMS. The determination of chloramphenicol in water, skimmed milk, honey, and urine samples was employed as example model to evaluate the proposal. Chloramphenicol is a broad-spectrum antibiotic very effective against both Gram-positive and Gram-negative bacteria. It was widely used to treat human and animal infections. However, it is not authorized in the EU since 1994 for use in food-producing animals due to its association with the induction of aplastic anemia. Nevertheless, chloramphenicol is still being used in the EU for human medicine and in treatments of non-food-producing animals [23]. Considering the evidences of in vivo carcinogenicity and genotoxicity of chloramphenicol, it is not appropriate to establish an acceptable daily intake, and maximum residue limits have neither been assigned for this antibiotic [24]. Screening methods are able to determine chloramphenicol residues at the minimum required performance limit (MRPL), established at $0.3 \mu\text{g kg}^{-1}$ and include diverse techniques like microbial inhibition tests, immunoassay, electrochemistry, and chromatography. Confirmatory methods, typically based on chromatographic methods coupled to mass spectrometry detectors, provide limits of quantification (LOQs) in the range of 0.01 to $0.3 \mu\text{g kg}^{-1}$ [23]. However, the main drawbacks of the aforementioned methodologies are the long analysis time required and the instrumentation cost. Thus, a logical analytical scheme for the detection of chloramphenicol residues in foods would be based on the application of rapid screening methods, with appropriate sensitivity and selectivity, followed by the confirmation of positive samples by liquid chromatography-tandem mass spectrometry.

Material and methods

Material, reagents, and samples

Cyanogen bromide (CNBr) activated Sepharose-4B from GE Healthcare Biosciences (Uppsala, Sweden) was used as solid support. Polypropylene SPE tubes (3 mL) with polyethylene frits ($20 \mu\text{m}$ porosity) were obtained from Scharlab (Barcelona, Spain). SupelMIP chloramphenicol molecular imprinted polymer was obtained from Supelco (Bellefonte, PA, USA).

Chloramphenicol (2,2-dichloro-N-[1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]acetamide) pure standard was purchased from Sigma-Aldrich (St. Louis, MO, USA) and stock solutions were prepared in 2-propanol and kept at 4°C in amber glass vials. Chloroform, methanol, 2-propanol, and buffer constituents were also obtained from Scharlab.

Skimmed milk and honey samples were obtained from the Spanish market and urine samples were obtained from different healthy volunteers, after appropriate information and oral consent. All the samples were stored at 4°C in the fridge until analysis. A lyophilized, partially defatted, raw bovine milk reference material (MI1413-2/CM), acquired from TestVeritas S.R.L. (Padova, Italy) with a certified chloramphenicol concentration of $1.56 \mu\text{g kg}^{-1}$ ($n = 11$) and a satisfactory range from 0.24 to $2.88 \mu\text{g kg}^{-1}$, was analyzed to assess the trueness of the methodology.

IAC procedure

The anti-chloramphenicol monoclonal antibody was produced in our laboratory following procedures for hybridoma generation [25]. Antibody was purified by ammonium sulfate precipitation and protein G affinity chromatography, being later fully characterized by enzyme-linked immunosorbent assay (ELISA). IAC columns were home-made produced by covalent immobilization of the antibody on Sepharose gel following previous studies [26]. IAC columns were produced using 5 mg antibody per gram of Sepharose gel, which is equivalent to 0.5 mg monoclonal antibody per column.

The IAC column was equilibrated with 5 mL phosphate buffer saline (PBS) plus 5 mL deionized water under gravity flow (approximately 1 mL min^{-1}). Samples (from 1 to 20 mL) were loaded through the column, washed with 2 mL deionized water, smoothly dried by passing air through the IAC by means of an empty syringe, and eluted with 1 mL 2-propanol. Honey samples (from 1 to 20 g) were dissolved using 2 mL deionized water per gram of honey prior to their extraction and analysis in order to improve fluidity through the column.

A volume of the elution solution was extracted by dispersive liquid-liquid microextraction (DLLME) in order to pre-concentrate chloramphenicol, but also to change the solvent for increasing the sensitivity of the IMS measurement. Thus,

taking into consideration the residual water amount of the elution fraction (30 % v/v), 150 μL of elution solution was mixed with 450 μL water and 50 μL chloroform as extraction solvent, in order to maintain the same water/2-propanol/chloroform ratio than that used in the aforementioned DLLME procedure. A cloudy solution was formed that was stable for a long time. Then, the mixture was centrifuged for 2 min at 5000 rpm and 10 μL of the chloroform extract (sedimented in the bottom of the conical tube) was directly injected and analyzed by IMS. The concentration of chloramphenicol in samples was calculated by interpolating the IMS signal a calibration curve prepared in the same conditions than samples using the DLLME procedure.

MIP procedure

A commercially available MIP specific for chloramphenicol, with a bed weight of 25 mg, was employed as SPE support. MIP was equilibrated with 5 mL 2-propanol plus 5 mL deionized water under gravity flow. Samples (from 1 to 20 mL) was loaded through the column, washed with 2 mL 2-propanol 20 % (v/v) in deionized water, completely dried using vacuum, and eluted with 1 mL 2-propanol. Honey samples (from 1 to 20 g) were likewise dissolved using 2 mL deionized water per gram of honey to increase sample fluidity. The MIP elution fraction was also extracted by DLLME, using 100 μL elution solution plus 500 μL water and 50 μL chloroform, to maintain the optimized solvent ratios. The concentration of chloramphenicol in samples was calculated by interpolating the IMS signal in a calibration curve prepared using the DLLME procedure.

IMS determination

An IONSCAN-LS from Smiths Detection (Morristown, NJ, USA) equipped with a ^{63}Ni foil radioactive ionization source was used for the IMS determination of chloramphenicol. IM station software (version 5.389) was used for plasmagram acquisition and data processing. Plasmagrams were acquired in negative ion mode using 4-nitrobenzotrile, with a reduced mobility (K_0) of $1.652\text{ cm}^2\text{ V}^{-1}\text{ s}^{-1}$, as internal calibrant and hexachloroethane, with a K_0 of $2.261\text{ cm}^2\text{ V}^{-1}\text{ s}^{-1}$, as ionization reactant. The number of segments per analysis was 120, with 20 co-added scans per segment and every plasmagram containing 479 data points. The shutter grid width was 0.2 ms, the value optimized by the manufacturer, and plasmagrams were collected with a scan period of 30 ms. A counterflow of dry nitrogen, set at 350 mL min^{-1} , was introduced as drift gas at the end of the drift region. The electric field strength in the drift region was 287 V cm^{-1} , with a total drift voltage of 2008 V and a drift tube length of 7 cm.

Sample introduction was performed by thermal desorption from a polytetrafluoroethylene (PTFE) membrane, using

10 μL injection volume. Desorption, inlet, and drift tube temperatures were adjusted to 255, 260, and 111 $^\circ\text{C}$, respectively. The sample tray, containing the PTFE membrane, was inserted in the heated zone using a 1-s post-dispense delay and the sample was held in this position for 60 s. Thus, the total analysis time for IMS acquisition was 1 min.

Reference UHPLC-MS procedure

Ultra high-performance liquid chromatography (UHPLC) coupled to a triple quadrupole mass spectrometer was selected as the reference methodology in order to obtain maximum sensitivity and selectivity in the analysis of chloramphenicol residues. UHPLC was performed on a Waters Acquity UPLC system from Waters (Milford, MA, USA), equipped with a binary solvent delivery system, an autosampler, and a BEH C18 (1.7 μm , $2.1 \times 50\text{ mm}$) column. An injection volume of 5 μL of the IAC extracts was directly analyzed by UHPLC-MS. The mobile phase consisted of (A) 0.1 % (v/v) formic acid in water and (B) methanol. The gradient started at 5 % of B was linearly increased to 95 % in 4 min, and then maintained 1 min at a flow rate of $0.4\text{ }\mu\text{L min}^{-1}$. The obtained retention time under the aforementioned conditions of chloramphenicol was 3.44 min. Tandem mass acquisitions were performed in a Waters Acquity triple quadrupole mass spectrometry detector, equipped with a Z-spray electrospray ionization source, with 3.5 kV capillary voltage, 120 $^\circ\text{C}$ source temperature, and 350 $^\circ\text{C}$ desolvation temperature. The employed parameters were as follows: ESI+, parent ion 321 m/z and daughter ions 257 and 152 m/z , using 25 V cone energy, and 15 and 25 eV collision energy, respectively.

Results and discussion

IMS plasmagrams of chloramphenicol

The ion mobility plasmagram of a chloramphenicol standard solution in 2-propanol is depicted in Fig. 1. The peaks present in the early portion of the plasmagram between 8 and 14 ms, which also occur in the blank injections, are product ions from IMS reactant and calibrant compounds. In negative ion mode, a chloride source (hexachloroethane) was used as reactant, and after the initial formation of negatively charged chloride, additional collisions lead to stable secondary ions, the most common of which is $\text{Cl}^-(\text{H}_2\text{O})_n$. It can react with oxygen of air, used as drift gas, which provided stable secondary ions such as $\text{O}_2^-(\text{H}_2\text{O})_n$. In summary, the hexachloroethane reactant and the 4-nitrobenzotrile calibrant yields alternate reactant ions such as Cl^- , NO_3^- , or NO_2^- , resulting in a complex signal, between 8 and 14 ms difficult to be analyzed [27].

Plasmagrams of chloramphenicol presented a main peak at $16.90 \pm 0.05\text{ ms}$ drift time (K_0 of $1.221 \pm 0.005\text{ cm}^2\text{ V}^{-1}\text{ s}^{-1}$)

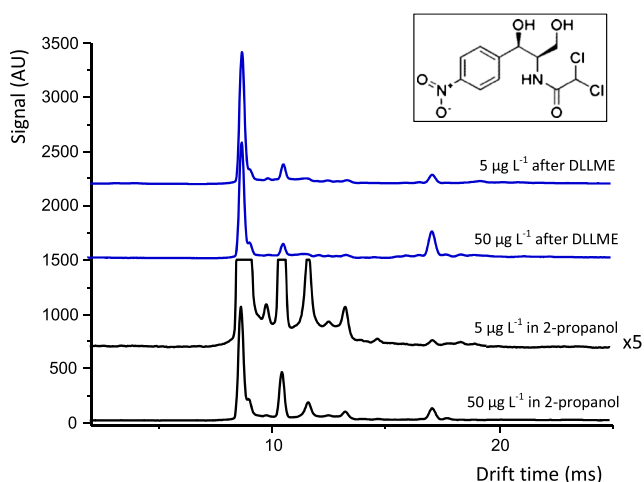


Fig. 1 Ion mobility spectrometry (IMS) plasmagrams of 5 and 50 $\mu\text{g L}^{-1}$ chloramphenicol standards dissolved in 2-propanol and measurements obtained after a dispersive liquid-liquid microextraction (DLLME) with water and chloroform. *Inset*, chemical structure of chloramphenicol

and a second smallest peak at 17.55 ± 0.02 ms (K_0 of 1.079 ± 0.002 $\text{cm}^2 \text{V}^{-1} \text{s}^{-1}$). Accordingly, the reported chloramphenicol K_0 value is consistent with previously published values (1.22 ± 0.05 $\text{cm}^2 \text{V}^{-1} \text{s}^{-1}$) [28].

An alarm was generated in the IMS software to alert for the presence of chloramphenicol in the samples, using the following peak descriptors: (i) K_0 value of 1.221 $\text{cm}^2 \text{V}^{-1} \text{s}^{-1}$, (ii) a variability value of 75 μs of the peak drift time, to compensate for small changes of the expected K_0 value, (iii) a full width value at the half-maximum height (FWHM) of the peak of 200 μs , (iv) the width of the peak must be no more than 1.5 times the programmed FWHM, and (v) a signal higher than the signal threshold value (10 arbitrary units). The total analysis time of chloramphenicol determinations by IMS was set at 30 s.

Analytical features of chloramphenicol determination by IMS

The height of the peak at 16.90 ms, obtained from the average plasmagram of a 3-s window around chloramphenicol desorption time, was employed as analytical response. Determination coefficient and linear range of the procedure were checked using calibration curves of chloramphenicol dissolved in 2-propanol and chloroform from 10 to 200 $\mu\text{g L}^{-1}$ using seven concentration levels. The obtained slopes were statistically comparable, independently of the solvent used, and the main analytical figures of merit are shown in Table 1. The instrumental limits of detection (iLOD) and quantification (iLOQ) were calculated as three and ten times, respectively, the standard deviation of the intercept divided by the slope of the calibration line, obtaining values of 10 and 30 $\mu\text{g L}^{-1}$, respectively. However, the obtained sensitivity was not enough to reach the MRPL of chloramphenicol in food

(0.3 $\mu\text{g L}^{-1}$) [23]. Accordingly, a pre-concentration of sample was carried out in order to improve the sensitivity of the method.

Development of IAC-IMS procedure

Chloramphenicol IAC columns were produced by using high specific monoclonal antibodies coupled to CNBr-activated Sepharose gel. The affinity of anti-chloramphenicol antibodies was characterized by enzyme-linked immunosorbent assay using a conjugate immobilized indirect assay with an average IC_{50} value of 3.2 $\mu\text{g L}^{-1}$. IAC columns were produced using 5 mg antibody per gram of Sepharose gel as proposed in previous studies [26]. Thus, the antibody was covalently immobilized on 100 mg of Sepharose gel with a coupling efficiency close to 100 %.

The most appropriate washing solution was established to provide an adequate sample clean-up and no analyte elution from the column. Several mixtures of 2-propanol in water, from 0 to 100 % (v/v), were evaluated to check the release of chloramphenicol from the IAC column. Therefore, IAC columns were loaded with 100 ng chloramphenicol (1 mL of 100 $\mu\text{g L}^{-1}$ standard), washed with 1 + 1 mL of 2-propanol from 0 to 100 % (v/v) in water, and eluted with 1 + 1 mL 2-propanol. The obtained results are shown in Fig. 2, where it can be seen that chloramphenicol began to be detected in the washing solution at 2-propanol concentration equal or higher than 5 % (v/v). Accordingly, pure water was selected as washing solution in order to avoid analyte losses during the washing step. Pure 2-propanol was selected as the most appropriate elution solvent taking into consideration the chloramphenicol quantitative elution in the first 1 mL volume and the compatibility with IMS detection.

Once SPE procedure was defined, chloramphenicol IAC columns were characterized in terms of analyte binding capacity and reusability of the immunosorbent. Columns were loaded with 10 μg of chloramphenicol (2 mL of 5 mg L^{-1} standard), washed with 2 mL water, and eluted with 1 mL 2-propanol, with an obtained binding capacity of 1.01 ± 0.15 μg chloramphenicol. The column reusability was evaluated by repeated recovery studies using spiked samples. Recovery values were calculated for ten repeated cycles using an IAC column loaded with 1 mL of water spiked at 50 $\mu\text{g L}^{-1}$ chloramphenicol in independent studies. Quantitative recoveries, between 85 and 122 % with RSD values lower than 10 %, were obtained in all cases; therefore, the same column can be reused at least ten times.

The amount of water in the elution fraction from IAC columns affected the chloramphenicol IMS signal intensity, compromising sensitivity of the methodology (see Electronic Supplementary Material (ESM) Fig. S1). Thus, the use of a DLLME was proposed in order to (i) remove water and (ii) pre-concentrate the MIP extract. Therefore, after the IAC-SPE

Table 1 Analytical figures of merit of the chloramphenicol determination by IMS analysis for standard solutions prepared in different solvents measured directly, and after solid-phase extraction using immunoaffinity chromatography (IAC) and molecular imprinted polymer (MIP)

Parameter	IMS	IAC-IMS	MIP-IMS
Standard solvent	2-Propanol chloroform	70 % (v/v) 2-propanol	2-Propanol
DLLME standard volume (μL)	–	150	100
Linear range ($\mu\text{g L}^{-1}$)	30–150	1.3–80.0	1.9–125.0
R^2	0.991	0.997	0.994
iLOD ($\mu\text{g L}^{-1}$) ^a	10	0.4	0.6
iLOQ ($\mu\text{g L}^{-1}$) ^a	30	1.3	1.9
MQL ($\mu\text{g L}^{-1}$) ^b	–	0.06	0.09
RSD (%) ^c	3.1/4.2 ^d	2.1	2.3

^a Calculated as three (instrumental limit of detection, iLOD) and ten times (instrumental limit of quantification, iLOQ) the standard deviation of the intercept divided by the slope of the respective calibration curve

^b Method quantitation limit (MQL). Calculated from the instrumental LOQ and considering an enrichment factor of 20

^c Calculated from three independent measurements of a chloramphenicol standard solution at LOQ level

^d RSD value was different for 2-propanol and chloroform standard solution

procedure, 150 μL of the eluted fraction was extracted by DLLME using 450 μL deionized water and 50 μL chloroform in order to maintain the optimized ratio of solvents (see ESM

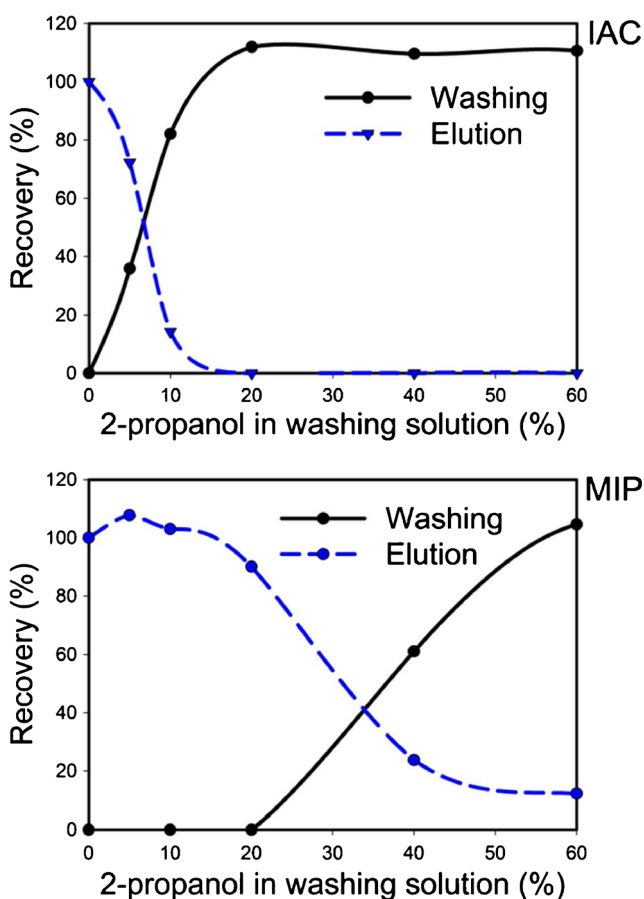


Fig. 2 Elution profile of chloramphenicol using different percentages of 2-propanol in water as washing solution and pure 2-propanol as elution solvent through immunoaffinity chromatography (IAC) and molecular imprinted polymer (MIP) solid-phase extraction. Columns were loaded with 100 ng chloramphenicol

Fig. S2). In the same way, a calibration line was prepared using 70 % (v/v) 2-propanol and extracted by DLLME. Table 1 shows the main analytical figures of merit of the IAC-IMS procedure regarding linearity, linear range, precision, iLOD, and iLOQ values. The total time for an IAC-IMS analysis of a sample was set in less than 10 min, and several samples (6–8) can be easily run in parallel.

Trueness of the IAC-IMS method was established by the evaluation of recoveries on water, skimmed milk, urine, and honey spiked with chloramphenicol at four concentration levels (0.1, 0.5, 5.0, and 50.0 $\mu\text{g L}^{-1}$). Different sample amounts were loaded on the IAC column depending on the spiked level, in order to include it inside the IMS linear range, and recoveries were determined (see Table 2). The obtained values ranged from 91 to 96 % for water, from 99 to 110 % for skimmed milk, from 88 to 104 % for honey, and from 95 to 116 % for urine. Thus, method quantitation limit (MQL) was set at 0.06 $\mu\text{g L}^{-1}$ for all evaluated matrices. Figure 3 shows the IMS plasmagrams of the four abovementioned matrices spiked at 50 $\mu\text{g L}^{-1}$ chloramphenicol. Chloramphenicol residues were accurately calculated for all samples evaluated till concentrations of 0.1 $\mu\text{g L}^{-1}$; thus, IAC methodology can be satisfactorily employed as screening tool for regulatory purposes.

Development of MIP-IMS procedure

A MIP procedure was developed employing commercial MIP supports specific for chloramphenicol. First of all, the adequate washing and elution solution was established as aforementioned for IAC supports. MIP column was loaded with 100 ng chloramphenicol, washed with different 2-propanol/water mixtures (from 0 to 100 % (v/v)), eluted with pure 2-propanol, and then analyzed by IMS. Figure 2 shows the obtained results, where it can be seen that a 20 % (v/v) 2-

Table 2 Recovery values for chloramphenicol spiked in water, skimmed milk, honey, and urine samples analyzed by solid-phase extraction followed by dispersive liquid-liquid microextraction using immunoaffinity chromatography (IAC) and molecular imprinted polymer (IAC) before their analysis by IMS

Method	[Spiked] ($\mu\text{g L}^{-1}$)	Sample amount ^a (mL)	Recovery (% \pm s, $n = 3$)			
			Water	Skimmed milk	Honey	Urine
IAC-IMS	50.0	1	96 \pm 8	102 \pm 7	88 \pm 3	99 \pm 7
	5.0	5	91 \pm 4	99 \pm 3	90 \pm 7	96 \pm 4
	0.5	10	94 \pm 10	102 \pm 7	104 \pm 8	95 \pm 6
	0.1	20	96 \pm 4	110 \pm 5	96 \pm 9	116 \pm 8
MIP-IMS	50.0	1	113 \pm 10	95 \pm 11	47 \pm 12	101 \pm 8
	5.0	5	108 \pm 3	120 \pm 10	43 \pm 23	117 \pm 11
	0.5	10	104 \pm 8	90 \pm 9	–	124 \pm 14
	0.1	20	123 \pm 11	111 \pm 12	–	104 \pm 18

^a One, 5, 10, and 20 g of honey were diluted in 2, 10, 20, and 40 mL deionized water, respectively

propanol in water could be employed as washing solution without elution of chloramphenicol. Pure 2-propanol was employed as elution solvent and the SPE support was taken to dryness, so no residual water was found in this case in the elution fraction by ATR-FTIR analysis. DLLME of the MIP-SPE extract was performed using 100 μL of the eluted fraction, 500 μL deionized water, and 50 μL chloroform to maintain the adequate ratio of solvents (see [ESM](#)). [Table 1](#) shows the main analytical figures of merit of the MIP-IMS procedure carried out on chloramphenicol standards prepared in 2-propanol. The total analysis time was scarcely higher (12 min) than that employed for IAC-IMS, including the vacuum drying step. Likewise, several samples can be extracted in parallel in order to increase the sample throughput.

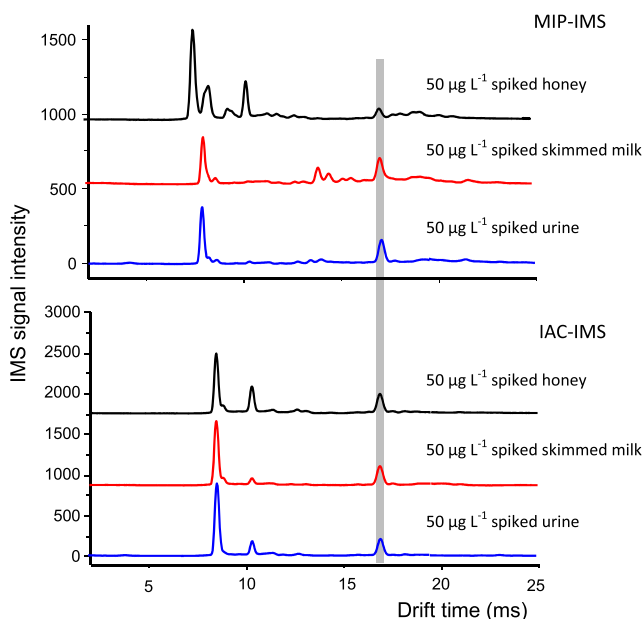


Fig. 3 IMS plasmagrams of urine, skimmed milk, and honey samples spiked with chloramphenicol at $50 \mu\text{g L}^{-1}$ using solid-phase extraction through immunoaffinity chromatography (IAC) and molecular imprinted polymer (MIP) as sample preparation step before ion mobility spectrometry (IMS) analysis

MIP columns were characterized similarly to IAC supports, in terms of chloramphenicol binding capacity (higher than $10 \mu\text{g}$) and reusability (until ten times). The MIPs presented a higher binding capacity than IAC supports as it is conventionally generalized, due to its higher number of active sites [29]. Recoveries were performed in water, skimmed milk, urine, and honey samples spiked with 0.1, 0.5, 5.0, and $50.0 \mu\text{g L}^{-1}$ chloramphenicol. [Table 2](#) shows the obtained recoveries for the different matrices. Quantitative recoveries were obtained for water (from 91 to 96 %), skimmed milk (from 90 to 120 %), and urine (from 101 to 124 %). The MQL values for these matrices were set at $0.09 \mu\text{g L}^{-1}$. Concerning honey samples, recoveries were lower than 50 % due to the presence of matrix compounds in the SPE extract, which strongly interfere the IMS analysis, as it can be seen in [Fig. 3](#). In any case, modifications of the clean-up procedure of the MIP-IMS procedure would be recommended to avoid interferences from non-specifically bounded compounds which interfere in the chloramphenicol determination of highly complex matrix, without dramatically affecting the global performance of the method in terms of low recoveries and sample throughput.

Critical comparison IACs vs MIPs

The potential of IAC and MIP columns was evaluated through the comparison of both procedures in terms of precision, recoveries, robustness, MQL, binding capacity and reusability, acquisition costs, and selectivity, among others.

As it can be seen in [Table 3](#), precision, robustness, and MQL values provided by IAC and MIP treatments were comparable. On the other hand, recoveries close to 100 % were obtained by IAC-IMS in all the matrices evaluated. However, honey samples presented important interferences that preclude chloramphenicol determination after MIP-IMS.

The high selectivity brought by the IAC cartridge was superior to the selectivity provided by the MIP cartridge for all the matrices evaluated (see [Fig. 3](#)). Compared with the signal obtained after IAC treatment, additional signals can be observed in the

Table 3 Critical comparison of immunoaffinity chromatography (IAC) and molecular imprinted polymer (MIP) as selective sorbents for chloramphenicol determination by IMS

Parameter	IAC-IMS	MIP-MIP
Robustness	Adequate	Adequate
Binding capacity ($\mu\text{g column}^{-1}$)	Low (≈ 1)	High (>10)
Reusability	Medium (≈ 10)	Medium (≈ 10)
Acquisition costs	High	Medium-low
Selectivity	Very good	Good

plasmagram of chloramphenicol spiked at the level of $50 \mu\text{g L}^{-1}$ in urine, skimmed milk, and honey samples after clean-up with MIPs, probably due to unspecific interactions between interferents and polymer. Regarding binding capacity, IAC cartridges showed a ten times lower value than that of the MIP, being comparable the reusability of both types of cartridges.

MIPs have demonstrated to be very stable at high temperatures, higher than $120 \text{ }^\circ\text{C}$, and treatments with organic solvents, strong acids and bases, being the IACs unstable with organic solvents, pH extremes, and at high temperatures. Although, the possible leaching of the MIP polymer during use is an important aspect to consider during method development. The commercial availability of MIP columns for a wide range of analytes is higher than that of IAC sorbents which should be custom-made developed for the analysis of new analytes. Moreover, development of antibodies may take several months, in front of the production period of MIPs that is from days to weeks. Regarding acquisition costs, MIPs have a relative low cost as compared to monoclonal antibodies employed in the production of IACs, which requires several phases like hapten design and synthesis, immunization of animals, production and clonation of hybridoma, and large scale production of antibodies [30].

In summary, although IACs are relatively expensive, the higher selectivity in front of MIPs and the quantitative recoveries obtained for all the evaluated matrices make IAC the preferred technique to improve the selectivity and sensitivity of IMS. So, IAC-IMS was selected for the analysis of chloramphenicol in food and clinical samples.

Validation of the proposed procedure

Analytical figures of merit of the proposed IAC-IMS methodology are shown in Table 1 and it was discussed in “Development of IAC-IMS procedure” section. Additionally, the extraction efficiency of IAC sorbents and trueness of the whole method was also evaluated by the analysis of a milk reference material with a certified chloramphenicol content of $0.24\text{--}2.88 \mu\text{g L}^{-1}$. The lyophilized milk sample was reconstituted in 9.30 g of distilled water, following the manufacturer recommended procedure. After the reconstitution, it

was stored at $4 \text{ }^\circ\text{C}$ overnight and used the day after. Before using the reference sample, it was heated in water bath at $40 \text{ }^\circ\text{C}$ up to the complete dissolution of all the clots. Therefore, it was cooled at room temperature and gently shaken. The aforementioned solution was analyzed using the IAC-IMS procedure in two independent replicates. The chloramphenicol concentration found using the proposed method was $1.12 \pm 0.07 \mu\text{g L}^{-1}$, which is included in the range of concentration certified by the supplier, indicating the agreement of the proposed procedure with the certified reference value.

Trueness of the IAC-IMS method was also assessed by method comparison using UHPLC-MS/MS as reference methodology for the analysis of chloramphenicol. A total of 32 samples, including 12 milk, 12 honey, and 8 urine samples, were extracted by using the proposed IAC method and analyzed by both, IMS and UHPLC-MS/MS methodologies. Chloramphenicol was not detected in any of them; thus, four milk, three honey, and five urine blank-samples were blind-spiked at concentrations from 0.1 to $2.0 \mu\text{g L}^{-1}$ chloramphenicol. Concentration values found by the proposed IMS procedure were compared with those obtained by the reference UHPLC-MS/MS method and they were in good accordance as it can be seen in Table 4, with bias values between -17.6 and 18.2% . Deming regression, which considers the standard deviation of both methods, was employed to evaluate the agreement between chloramphenicol concentrations obtained by the reference UHPLC-MS/MS and proposed IMS methods. Comparison of results afforded a regression with a slope of 0.94 (95 % confidence interval was from 0.88 to 1.01) and an intercept of 0.015 (95 % confidence interval was from -0.033 to 0.064), indicating that results obtained by both procedures were statistically comparable.

Table 4 Analysis of chloramphenicol blind-spiked samples at different concentration levels using the developed immunoaffinity chromatography extraction by IMS and ultra high-performance liquid chromatography-tandem mass spectrometry methods

Sample	[Chloramphenicol] ($\mu\text{g L}^{-1} \pm s$)			Bias (%)
	Blind-spiked	IAC-IMS	IAC-UHPLC-MS/MS	
Milk	2.00	1.81 ± 0.12	1.89 ± 0.09	4.2
	1.00	0.91 ± 0.09	0.86 ± 0.07	-5.8
	0.50	0.45 ± 0.16	0.45 ± 0.08	0.0
	0.20	0.21 ± 0.04	0.22 ± 0.05	4.5
Urine	1.00	0.99 ± 0.03	1.05 ± 0.04	5.7
	0.50	0.49 ± 0.04	0.46 ± 0.06	-6.5
	0.30	0.27 ± 0.05	0.29 ± 0.03	6.9
	0.20	0.20 ± 0.02	0.17 ± 0.05	-17.6
Honey	0.10	0.09 ± 0.02	0.11 ± 0.04	18.2
	1.00	0.82 ± 0.12	0.97 ± 0.08	15.5
	0.50	0.43 ± 0.07	0.41 ± 0.06	-4.9
	0.10	0.12 ± 0.02	0.11 ± 0.03	-9.1

Conclusion

IMS determination provides a rapid and high sensitive determination of organic compounds in simple matrices, but the analysis of complex ones cannot be made directly and a highly selective sample treatment is required. In this study, IAC and MIP have been evaluated and compared for the analysis of traces of chloramphenicol in food and urine samples by IMS. Excellent results, in terms of sensitivity, reusability of columns, and sample throughput, were obtained for both of them. However, MIP treatment provided honey extracts much more dirties than those obtained using IAC, thus presenting important interferences. Consequently, the off-line coupling of selective IAC supports and DLLME with IMS analysis offers an attractive strategy to obtain sensitive and selective analysis of chloramphenicol residues, which can be useful in food safety monitoring studies with a MQL value of $0.06 \mu\text{g L}^{-1}$, which is lower than the MRPL established by the EU.

In summary, IAC-IMS can be considered as an interesting and serious alternative to available screening methods based on immunoassays, reducing the number of false positive samples and providing quantitative data. On the other hand, the IAC-IMS method is simple, cost affordable, and does not required skilled operators to obtain accurate and precise results compared to alternative methods based on UHPLC-MS/MS. Moreover, a minimal consumption of organic solvents was required and it only used 1 mL 2-propanol and 50 μL chloroform per sample. Thus, it can be considered an environmentally friendly methodology that follows the principles of green analytical chemistry and it provides results statistically comparable to chromatographic reference methods. Nevertheless, the proposed IAC-IMS methodology provides a high specificity but only for a target analyte and in the case of multiresidue determination the acquisition and optimization of additional selective sorbents is required.

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Compliance with ethical standards This study has been approved by the appropriate ethics committee and has been performed in accordance with the ethical standards.

Conflict of interest The authors declare no competing financial or non-financial interest.

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