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Determination of chloramphenicol residues in fish meats by liquid chromatography–atmospheric pressure photoionization mass spectrometry

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Abstract

A liquid chromatography–atmospheric pressure photoionization (APPI) mass spectrometry method was developed for the determination of chloramphenicol (CAP) in fish meats (young yellowtail and flatfish). For the optimization of APPI, several APPI ion source parameters and mobile phases were investigated. CAP with APPI using the optimized parameters gave simple mass spectra and a strong signal corresponding to $[M-H]^-$ was observed. Further, APPI was compared with atmospheric pressure chemical ionization (APCI) and APPI gave similar results in terms of structural information and a better signal-to-noise ratio. The samples were extracted with acetonitrile and evaporated to dryness followed by a clean-up step using the liquid–liquid distribution between acetonitrile and *n*-hexane. The mean recoveries of chloramphenicol from a young yellowtail meat and a flatfish meat spiked at 0.1–2 ng/g were 89.3–102.5 and 87.4–94.8%, respectively. The limit of detection (signal-to-noise ratio=3) of the young yellowtail meat and the flatfish meat were 0.1 and 0.27 ng/g
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Keywords: Atmospheric pressure photoionization; Fish; Interfaces, LC–MS; Food analysis; Chloramphenicol; Antibiotics

1. Introduction

Chloramphenicol (CAP) is a broad-spectrum antibiotic, exhibiting activity against a variety of aerobic and anaerobic microorganisms. Its action works through interference with/or inhibition of protein synthesis. However, weeks or months of CAP therapy can result in a well-understood and irreversible type bone marrow depression called aplasia or hypolasia. This in turn can lead to aplastic anemia and although uncommon, it is often fatal. Because of these health concerns, a joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives has proclaimed that residues of CAP in the human food supply are unacceptable [1]. The use of CAP in food products has been banned in the European Union (EU) and the USA. However, CAP's broad spectrum

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of activity, ready availability and low cost, attract its use by some third world countries. Admittedly, whenever CAP is accessible, indiscriminate and illegal use potentially exists. In fact, the presence of CAP has been detected in shrimps intended for human consumption and imported from China and Vietnam.

Thus, a sensitive and reproducible method for the determination of CAP at residual levels is urgently needed. Several analytical methods for the determination of CAP in different biological materials, based on gas chromatography–electron-capture detection (GC–ECD) [2,3], gas chromatography–mass spectrometry (GC–MS) [4,5], liquid chromatography–photodiode array detection (LC–DAD) [5–8] and liquid chromatography–mass spectrometry (LC–MS) [5,8–12]. However, for GC–ECD or GC–MS techniques, sensitivity is not withstanding and the presence of interfering peaks around the CAP was claimed making the methods highly controversial in terms of quantification and qualification. Therefore, LC seems to be a good alternative. Furthermore, hyphenated techniques such as LC coupled to MS detection have been intensively developed and applied in residual analysis in food. The high selectivity and high sensitivity of MS detection methods associated with the resolution of LC provide decisive advantages to perform qualitative as well as quantitative analysis of a wide range of molecules at trace levels. However, the coupling of both techniques is only possible using a suitable interface. Atmospheric pressure ionization (API) interfaces, represented by atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) are commonly used in LC–MS. APCI and ESI are affinity methods in which the analytes either protonate or form adducts for positive ion mode and deprotonate or attach electron for negative ion mode. These properties are generally associated with polar compounds. Common problems for ion molecular affinity ionization methods such as APCI and ESI are as follows

(i) The desired signal can be suppressed in APCI and ESI by compounds with higher charge affinities than the target compounds.
(ii) Adduct formation with trace constituents is prevalent by ESI.

On the other hand, atmospheric pressure photoionization (APPI) is a new alternative ionization technique for LC–MS [13–16]. The APPI source is based on a high-frequency gas discharge lamp that generates vacuum-ultraviolet (VUV) photons of 10 and 10.6 eV energy. The energy of this discharge lamp is normally greater than a first ionization potential (IP) of the analyte because many organic compounds have IPs in the range of 7–10 eV. On the other hand, the IPs of the most commonly used LC solvents as a mobile phase have the higher IPs (water, IP=12.6 eV; methanol, IP=10.8 eV; acetonitrile, IP=12.2 eV). Therefore, APPI may directly ionize only molecules of the analyte that has a relatively lower IP and may overcome the above mentioned problem for APCI and ESI. However, to the best of our knowledge it has never been applied for residual analysis in food.

This paper focuses on the optimization and the suitability of LC–MS using the APPI technique for the determination of CAP in fish meats.

2. Experimental

2.1. Chemicals

CAP was obtained from Sigma–Aldrich Japan (Tokyo, Japan). The purity of this compound was higher than 99%. Stock solutions at 1 mg/ml were prepared in methanol, stored in the dark at 4 °C and diluted to the desired concentrations prior to use. Ammonium acetate, pesticide-grade ethyl acetate, anhydrous sodium sulphate, acetonitrile, HPLC-grade methanol and *n*-hexane were obtained from Wako (Osaka, Japan). Pure water was purified with a Milli-Q system (Millipore, Tokyo, Japan). A nylon-type 0.22 μm centrifuge filter was obtained from Toyo Soda (Tokyo, Japan).

2.2. Liquid chromatography–mass spectrometry

An Agilent 1100 series liquid chromatograph (Agilent Technologies, Waldbronn, Germany), consisting of a vacuum solvent degassing unit, a binary high-pressure gradient pump, an automatic sample injector and a column thermostat was used for LC–MS analysis. Further a model Agilent 1100 series diode array detector (Agilent Technologies) was connected on line with the mass-selective detector

(MSD). LC separation was performed on a 150×3 mm I.D. column packed with 5 μm Zorbax eclipse XDB C_{18} (Agilent Technologies, Palo Alto, CA, USA) using a linear gradient from B–A (10:90) to B–A (30:70) in 15 min. Solvent A was water containing 10 m*M* ammonium acetate and solvent B was methanol. The flow-rate was set at 500 μl/min. Further, acetone was added after the diode array detector at a flow-rate of 50 μl/min via a tee by an isocratic pump (Agilent Technologies, Waldbronn, Germany).

Agilent 1100 series MSD single quadrupole mass spectrometer equipped with the orthogonal spray-APCI and APPI (Agilent Technologies, Palo Alto, CA, USA). Nitrogen as a nebulizer gas and a drying gas of the ion source was generated from pressurized air by a Whatman model 75-72 nitrogen generator (Whatman, Haverhill, USA). For APPI, the nebulizer gas, the drying gas, a capillary voltage for the ion transmission, a fragmentor voltage for in-source-fragmentation and a vaporizer temperature were set at 50 p.s.i., 7 l/min, 3500 V, 120 V and 350 °C, respectively (1 p.s.i. = 6894.76 Pa). On the other hand, for APCI, the nebulizer gas, the drying gas, the capillary voltage, the fragmentor voltage and the vaporizer temperature were set at 40 p.s.i., 5 l/min, 4000 V, 120 V and 350 °C, respectively. Skimer and entrance lens voltage in the ion source of the MS system were automatically optimized by calibrant delivery system using a calibration standard (Agilent Technologies, Palo Alto, CA, USA) at 0.1 ml/min and set to 23, 57 V for APPI and 31, 48 V for APCI, respectively. The LC–MS determination was performed by operating the MS system in the negative ion mode. Mass spectrum was acquired over a scan range m/z 100–400 using a step size of 0.1 u and a scan speed of 0.5 scan/s. Quantitative analysis was carried out using the selected ion monitoring (SIM) mode of a base ion peak at m/z 321 with a dwell time of 500 ms. To verify the presence of target analytes in fish meat, a halogen isotopic ion of CAP was monitored.

2.3. Sample preparation

The samples analysed (young yellowtail, flatfish) were obtained from a local market. Then, 5 g anhydrous sodium sulphate and 10 ml ethyl acetate were added to 5 g fish meat, weighted into a centrifuge tube. The mixture was homogenized for 20 s with an Ultra-Turrax TP 18/10 (Janke and Kunkel, Staufen, Germany). After centrifugation for 5 min at 6000 rev./min, the supernatant was removed and transferred to a round flask. This extraction step was repeated twice, each time with 10 ml ethyl acetate. The combined ethyl acetate extract was then rotated in a rotary evaporator at 40 °C under vacuum to evaporate to dryness. After that, 1 ml acetonitrile and 1 ml *n*-hexane were added the residue. The dissolved residue was transferred into a graduated glass stopped reagent bottle and shaken. The *n*-hexane phase was discarded. This step was repeated with a further 1 ml portion of *n*-hexane. Finally, the acetonitrile phase was evaporated to dryness under a stream of dry nitrogen using a heating block at 50 °C. The dry residue was redissolved in 0.5 ml of acetonitrile–water (10:90) containing 10 m*M* ammonium acetate and the redissolved solution was filtered through a 0.22 μm nylon centrifuge filter. Aliquots of 20 μl were injected on the LC column.

Calibration curves of CAP were constructed by LC–APPI-MS using SIM mode with CAP standard solutions at 0.1–100 ng/ml.

3. Results and discussion

3.1. Optimization of the APPI parameters

To optimize the APPI conditions, different parameters which influence the ionization efficiency and a mass spectrum were investigated for only APPI because APCI has already been investigated. The drying gas flow, the nebulizer gas pressure, the vaporizer temperature, the capillary voltage, the fragmentor voltage and the mobile phase were investigated under the chromatographic conditions mentioned in the experimental section by using a deprotonated molecular ion (m/z 321) as a target ion. However, the modification of the drying gas flow-rate and the nebulizer gas pressure did not drastically improve the sensitivity of CAP. Therefore, the effects of the fragmentor voltage, the capillary voltage, the vaporizer temperature and the mobile phase are described below.

3.1.1. APPI mass spectrum and effect of fragmentor voltage

There are three main means of producing an ion by APPI: (1) direct photoionization; (2) electron-transfer with dopant ion; or (3) proton transfer with a dopant or other protic gas phase species. APPI makes use of a photoionizable dopant added to the vapor generated from the eluant to increase the ionization efficiency of the molecules in the vaporized LC eluant. Acetone or toluene is usually used as the dopant. In this study, acetone was added into the APPI source after the column at a flow-rate of 50 μl/min.

The fragmentor voltage is applied to the exit of the capillary and affects the transmission and fragmentation of sample ions between the exit of the capillary and the skimmer at relatively high pressure (3 Torr; 1 Torr = 133.322 Pa) [17,18]. In general, the higher fragmentor voltage which helps the transfer of ions in the relatively high pressure region between the exit of the capillary and the skimmer, produces the more fragment ions. First of all, CAP was ionized by positive ion mode. However, hardly any signal is observed for $(M)^{+}$ radical cation or $(M+H)^{+}$ ion. This indicates that any direct ionization by photon did not occur. Therefore, CAP seems to have a high ionization potential (IP) or low proton affinity.

On the other hand, for the negative ion mode, the deprotonated molecular ion $(M-H)^{-}$ was observed as the predominant ion. The ionization mechanism of APCI with the negative ion mode is that CAP captures electrons generated from the corona discharge and will lose a proton to become negatively charged because CAP has high electron affinity. For APPI, the similar mechanism or the ion–molecule reaction with the ion in the mobile phase, which leads to the formation of the observed $(M-H)^{-}$ ion, seems to occur. It is, however, beyond the scope of this article to expound on the ion-molecule chemistry of the APPI source. Thus, to establish the optimum fragmentor voltage for the analysis of CAP in the negative ion mode, the intensity of this compound vs. the fragmentor voltage was studied in the range 80–200 V. As shown in Fig. 1, the optimum fragmentor voltage was found at 120 V, whereas a significant intensity reduction was observed at higher values. Further, the highest signal-to-noise (S/N) ratio was also observed at 120 V. The mass spectra

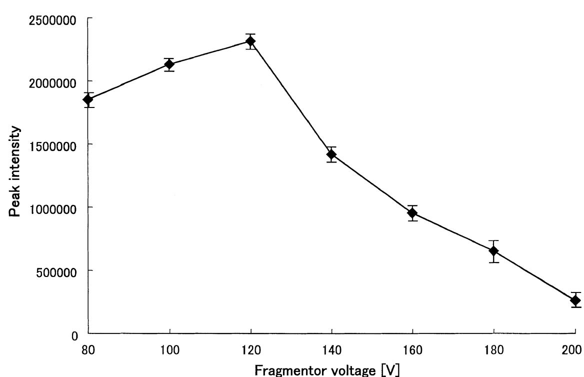

Fig. 1. The effect of the fragmentor voltage on the peak intensity of CAP. Concentration: 1 μg/ml. Averages of five duplicate measurements are represented. For other conditions, see the Experimental section.

of CAP at the optimal and higher fragmentor voltage were shown in Fig. 2. These mass spectra showed that the $(M-H)^{-}$ ion was the predominant ion at 120 V and this ion included isotopic ions (m/z 321, ^{35}Cl ^{35}Cl; m/z 323, ^{35}Cl ^{37}Cl; m/z 325, ^{37}Cl ^{37}Cl) because CAP includes two chlorines. On the other hand, the higher fragmentor voltage (180 V) generated structurally relevant fragment ions. The m/z 152 fragment ion giving the largest intensity might be generated from the cleavage of the carbon–carbon bond on the alkyl branch. Further, other fragment ions were

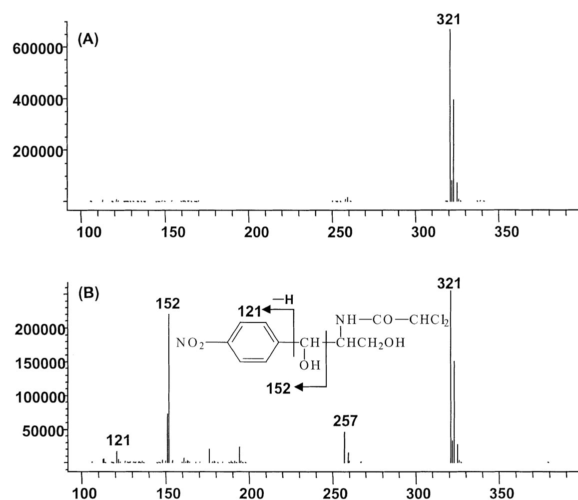

Fig. 2. The mass spectra of CAP at two different fragmentor voltage. (A) Low fragmentor voltage (120 V). (B) High fragmentor voltage (180 V).

observed at m/z 121 and 257 and they might be a nitrophenyl moiety and a fragment ion due to loss of a hydrochloric acid molecule and an aldehyde group from the alkyl branch, respectively. These fragment ions observed by APPI corresponded with the fragment ions by ESI [5] and APCI. Based on the above results, the fragmentor voltage was set at 120 V.

3.1.2. Effect of capillary voltage

The capillary voltage is applied to the inlet of the capillary and influences the transmission efficiency of the ions through a capillary sampling orifice. There is one additional feature of the APPI source: ion formation by APPI does not require any element within the ionization region at a high potential unlike ESI and APCI, where the spray needle and corona discharge needle are operated at several kV. Therefore, the APPI source is essentially field free. This characteristic allows for the independent optimization of the capillary voltage, which may have a tremendous effect upon the sensitivity of the method. To establish the optimum capillary voltage, this parameter was varied from 1000 to 4000 V. As shown in Fig. 3, 1500 V was found optimum and a tremendous effect of this parameter on the intensity of CAP was observed in the case where acetone was not used as the dopant. On the other hand, when acetone as the dopant was introduced into the APPI source, the maximum intensity of the ion was found at 3500 V and the intensity found at 3500 V with the dopant was a little higher than the maximum intensity without the dopant. Then, the S/N ratio of the CAP peak with the dopant was compared with one without the dopant by using the standard solution at the 1 ng/ml concentration. As a result, the S/N ratio with the dopant (S/N ratio=35) was higher than it without the dopant (S/N ratio=15) because the introduction of acetone as the dopant decreased background noise. The other important aspect is that the capillary voltage had the insignificant effect on the intensity by using the dopant and the ion current measured at the capillary inlet was much higher than it without the dopant. This result indicates that acetone as the dopant could generate enough electrons to improve the ionization efficiency and excess amount of electrons could discount differences of the capillary voltage. Based on the above results, acetone was introduced into the APPI source and the capillary voltage was set at 3500 V.

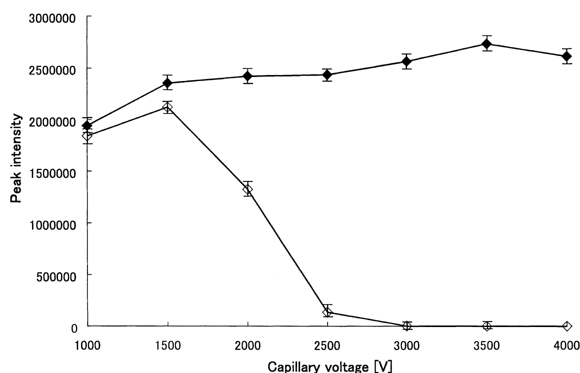

Fig. 3. The effect of the capillary voltage on the peak intensity of CAP. Concentration: 1 μg/ml. Averages of five duplicate measurements are represented. For other conditions, see the Experimental section. ◇, Without acetone as the dopant; ♦, with acetone as the dopant.

3.1.3. Effect of vaporizer temperature

For APPI, the vaporizer temperature plays a key role for the complete evaporation of CAP because ionization occurs in the vapor state like APCI. Thus, in case of using linear gradient elution, this temperature must be kept to the high temperature so that the change of mobile phase composition does not influence to the ion intensity of CAP. Under high temperature, however, the risk of the thermal degradation occurs. In this study, the vaporizer temperature was varied between 250 and 450 °C to optimize the intensity and the S/N ratio. As a result, the highest temperature for a maximum intensity and the S/N ratio of CAP was observed at 350 °C. The intensity of CAP decreased as the vaporizer temperature was set at over 400 °C and an intensive fragmentaion was observed in the mass spectrum at 400 °C. Therefore, the decrease of the intensity over 400 °C may be a result of the thermal degradation. Further, this experiment was done by the flow injection mode with the different composition of the mobile phase ranged from methanol–water (10:90) to methanol–water (70:30). The results showed that the vaporizer temperature of 350 °C generated the maximum ion intensity and the S/N ratio of CAP with all ranges. Based on the above results, the vaporizer temperature was set at 350 °C.

3.2. Optimization of the chromatographic conditions

The separation of CAP from sample matrix peaks was optimized using acetonitrile, methanol, water and ammonium acetate. The combination acetonitrile and ammonium acetate was found optimum for the separation of CAP. However, when methanol was replaced with acetonitrile, a significant signal decrease was observed and the S/N ratio also decreased. This result was different from APCI. As above mentioned, the concept that CAP captures electrons generated from the corona discharge and will lose a proton to become negatively charged in APCI can explains that the mobile phase does not influence the ionization efficiency. On the other hand, in the APPI negative mode, if an ionization mechanism like APCI would occur in the APPI source, methanol could generate much more electrons by photons because the IP of methanol is lower than that of acetonitrile and improve the sensitivity of CAP. Therefore, methanol and 10 m*M* ammonium acetate were used as the mobile phase considering the sensitivity in this study.

Generally speaking, APCI and APPI are mass-flow dependent ionization techniques unlike ESI which is a concentration-dependent ionization technique, because they are gas phase ionization methods. However, Yang and Henion reports that for neutral compounds an important observation with APPI is an apparent increase in sensitivity when the flow-rate is reduced [19]. Then, the influence of the mobile phase flow-rate on the intensity (the peak area) of CAP in APPI was investigated by varying from 0.2 to 1 ml/min. As the result, the maximum intensity of CAP was found at 0.5 ml/min and there occurred a drop of the intensity of CAP beginning at flow-rates higher than 0.5 ml/min. This result leads to the conclusion that the loss in intensity of CAP under high-flow conditions is mainly due to decreasing efficiency of the ionization process with increasing amount of eluent sprayed into the APPI source. If electrons are concerned in the ionization efficiency, the electron density might be affected by the flow-rate. However, the ionization mechanism has to be investigated in more detail for APPI negative ion mode. On the basis of this result, the flow-rate was set at 0.5 ml/min.

Table 1
Linearity, limit of detection and precision of CAP in standard solutions by APPI and APCI

Ionization mode	r^2	Limit of detection[a] (ng/ml)	Repeatability[b] (RSD, %)
APPI	0.9998	0.07	2.1
APCI	0.9996	0.09	4.5

[a] Detection limit is limit of detection defined as $S/N = 3$ at 0.1 ng/ml.
[b] Repeatability was calculated on the basis of five replicates at 0.5 ng/ml within 1 day.

3.3. Linearity, detection limit and precision of LC–APPI-MS and LC–APCI-MS systems

At first, the analytical performance characteristics of the optimized LC–APPI-MS and LC–APCI-MS methods were determined on standard solutions of CAP in pure solvent. In order to achieve maximum sensitivity, all experiments were carried out in the SIM mode using the mass corresponding to the $[M-H]^-$ ions of CAP. To test the linearity of the calibration curve, various concentrations of CAP in the range from 0.1 to 100 ng/ml were analyzed. As shown in Table 1, the calibration curves of both ionization modes showed good linearity with correlation coefficients (r^2) above 0.999. The repeatability of the both methods for a standard solution was calculated on the basis of five replicates at 0.5 ng/ml in the same day. The limit of detection (LOD) was calculated by using the S/N ratio of 3 at 0.1 ng/ml.

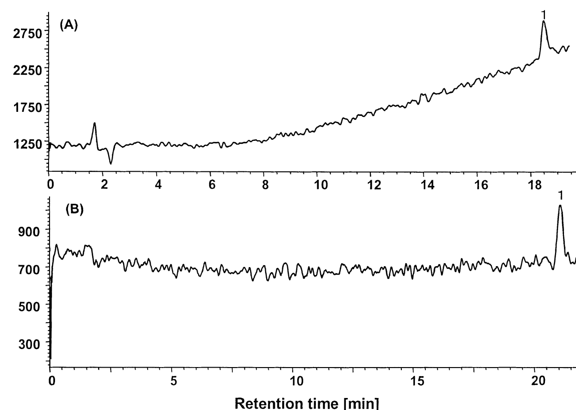

Fig. 4. SIM chromatograms of CAP in pure solvent at 0.1 ng/ml with APCI (A) and APPI (B). 1, CAP.

As shown in Table 1, LOD and RSD of CAP were 0.07 ng/ml, 2.1% and 0.09 ng/ml, 4.5%, respectively. These results indicated that the performance of APPI was very similar to those of APCI. Fig. 4 shows a comparison of the *S/N* ratio observed for the SIM chromatograms of CAP with APCI (Fig. 4A) and APPI (Fig. 4B). As can be seen from these data, APPI had similar *S/N* ratio (4.3) compared with the *S/N* ratio (3.1) of APCI. However, the chromatographic baseline with APPI was lower than that with APCI. This result suggests improved selectivity for APPI technique for CAP relative to chemical background in the system. This characteristic could afford some advantages for the analysis of food extracts since the analyte could perhaps be more easily detected in the presence of matrix components.

3.4. Comparison of APPI and APCI for the analysis of fish meat

The sample preparation involved the extraction step of CAP from fish meats using the homogenization step with ethyl acetate and sodium sulphate followed by the one clean-up step using the liquid–liquid distribution between acetonitrile and *n*-hexane. Generally speaking, affinity-based ionization technique, such as APCI and ESI, are susceptible to competition for charge effects due to some matrix components that coelute with analytes. Therefore, the matrix effect in the APPI and APCI determination was investigated by comparing SIM chromatogram obtained from standard solution in pure solvent with one obtained from matrix matched standard solution prepared from the analyte-free young yellowtail meat extract. As can be seen from the data in Fig. 5, it was observed that the young yellowtail meat matrix led to alternations in the chromatograms, which included some additional peaks and a rise in the base line in comparison with them in Fig. 4. However, intensities of these additional peaks and the baseline with APPI were lower than those with APCI and caused no interference. Furthermore, the changes on retention time and the peak intensity with APPI and APCI were 0.6, 5.5% and 0.7, 13.7%, respectively. These results indicate that APPI has the higher selectivity and the lower matrix effect than APCI. Then, a calibration curve in standard solutions in the range from 0.1 to 10 ng/ml was compared with one in young yellowtail meat extracts in the range from 1 to 100 ng/ml by using APPI. As presented in Table 2, there is no significant difference in calibration equations and correlation coefficients. These results indicate that it is possible to use external standards instead of matrix matched standards. This property of APPI is very useful for high-throughput applications because it minimizes the need to prepare matrix matched standards.

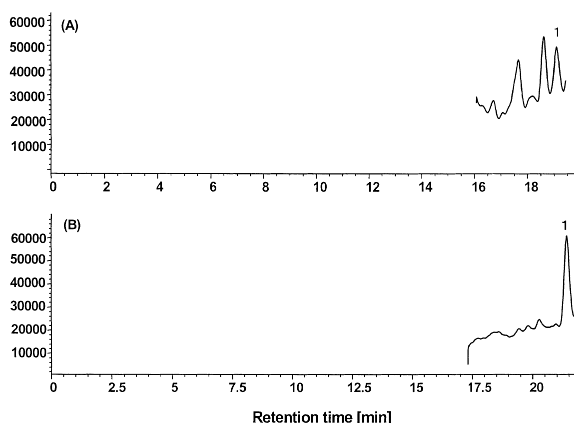

Fig. 5. SIM chromatograms of CAP in young yellowtail meat extract at 1 ng/g with APCI (A) and APPI (B). 1, CAP.

Another important observation with APCI is that the ion source could be contaminated and as a result, intensities of CAP could decrease over long time during the repeated analysis of fish meat extracts. Then, the feasibility of using APPI-MS and APCI-MS as detection methods for routine determination of CAP in fish meats was evaluated over 2 days. This investigation was performed by injecting the extract of the young yellowtail meat spiked at 1 ng/g. The resulting data showed that intensities of

Table 2
Linearity of calibration curves in a standard solutions and young yellowtail meat extracts

	Calibration equation $y = ax + b$[a]	r^2 [a]
Standard solution	$y = 63113x + 1274$	0.9998
Young yellowtail	$y = 68252x + 2453$	0.9997
Flatfish	$y = 66421x + 1743$	0.9995

[a] r^2 is correlation coefficient, x is the injected concentration in ng/ml and y is the peak intensity.

CAP by using APPI did not show any definite tendency to decrease over 2 days. The relative intensity of CAP in the last analysis to that in the first analysis with APPI was 91.3%. On the other hand, for APCI, the relative intensity of CAP in the last analysis was 52.3%. Thus, it appeared that a moderate contamination of the APPI source did not affect the ionization efficiency whereas a contamination of a corona needle in the APCI source decreased the ionization efficiency. From the above results, the developed APPI method was evaluated for the analysis of CAP residue in the fish meat because the APPI method was more suitable for the analysis of CAP in the fish meat.

3.5. APPI method evaluation

To evaluate recoveries, the proposed method was applied to the analysis of spiked CAP-free samples of young yellowtail and flatfish meat. Eighteen samples of two different fishes were each spiked with CAP, and each sample was spiked at three levels. The spiking levels ranged from 0.1 to 2 ng/g. Typical chromatograms from extracts of the blank fish meat and the fish meats spiked at 1 and 0.1 ng/g are shown in Fig. 6. Data from Eighteen spiked samples led to recoveries and RSD are summarized in Table 3. Mean recoveries ranged from 87.4 to 102.5% with RSD of 4.3 to 6.7%. The LOD of CAP in fish meats was determined by the signal corresponding to three times the background noise on SIM chromatogram of sample spiked at 1 and 0.1 ng/g. As shown in Table 4, the LODs of CAP in the young yellowtail meat and the flatfish meat were 0.27 and 0.1 ng/g, respectively. The intra-day precision (repeatability) was estimated by injecting the fish meat extract spiked at 0.5 ng/g five times during a working day. The inter-day precision (reproducibility) was evaluated by analyzing the same sample over five working days. The repeatability and reproducibility for CAP in young yellowtail meat and flatfish meat were 4.6, 7.9% and 3.2, 6.7%, respectively (Table 4). The quantitative results of CAP in both fish meats at 0.5 ng/g using external standard also are shown in Table 4. The mean accuracy of both fish meats was 14 and 8%. These results indicate that this LC–APPI-MS method using external standard is suitable for the analysis of CAP residues in fish meats.

Table 3
Recovery of CAP for spiked fish meat by APPI

Spiking levels (ng/g)	Recovery(±RSD (%))[a]	
	Young yellowtail	Flatfish
0.1	89.3±5.1	87.4±6.1
0.5	102.5±4.9	94.8±6.7
2.0	96.1±4.3	91.8±4.9

[a] Three spiked samples at the same amount were analysed.

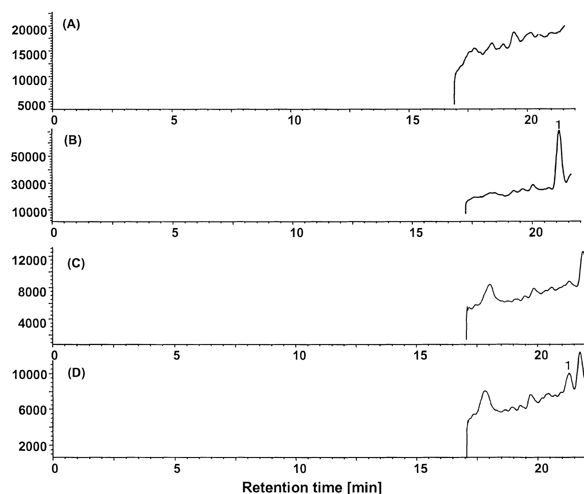

Fig. 6. SIM chromatograms of (A) a young yellowtail meat, (B) a spiked young yellowtail meat at 1 ng/g CAP, (C) a flatfish meat, and (D) a spiked flatfish meats at 0.1 ng/g CAP. 1, CAP.

In conclusion, the APPI technique was an ideal ionization technique because of high sensitivity and high selectivity for the determination of CAP in fish meats. An important advantage of using APPI for CAP content of fish meats is that matrix effect was not observed. Consequently, the proposed method eliminates the need of matrix matched standards, which is more tedious for the samples from different origins. Further, validation data demonstrate that this method is convenient for routine analysis of CAP

Table 4
LODs and precision of chloramphenicol in spiked young yellowtail meat and flatfish meat by APPI

Fish meats	LODs[a] (ng/g)	Repeatability[b] (RSD, %)	Reproducibility[c] (RSD, %)	Quantitative[d] results (ng/g)
Young yellowtail	0.27	4.3	7.9	0.43
Flatfish	0.10	3.2	6.7	0.46

[a] Detection limit is limit of detection defined as $S/N=3$ at 1 and 0.1 ng/g.
[b] Repeatability was calculated on the basis of five replicates at 0.5 ng/g within 1 day.
[c] Repeatability was calculated on the basis of single analysis per 1 day for 5 days at 0.5 ng/g.
[d] Calculated for five fish meats spiked at 0.5 ng/g.

residues in fish meats at trace levels, since excellent recoveries and repeatability for different samples were demonstrated.

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