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Development of an HPLC-MS/MS method for the quantitative determination of chloramphenicol in honey from Kazakhstan

Farida Baktybayeva*
Master of Technical and Biological Science, PhD Student
Kazakh National Agrarian Research University
050010, 8 Abay Ave., Almaty, Republic of Kazakhstan
https://orcid.org/0000-0002-6279-2731
Asiya Serikbayeva
Doctor of Biological Sciences, Professor
Kazakh National Agrarian Research University
050010, 8 Abay Ave., Almaty, Republic of Kazakhstan
https://orcid.org/0009-0005-8659-9230
Maxat Toishimanov
Master of Technical Science, Engineer
Kazakh National Agrarian Research University
050010, 8 Abay Ave., Almaty, Republic of Kazakhstan
https://orcid.org/0000-0002-6070-4574
Maksat Serikov
Master of Technical Science, Research Associate
Kazakh National Agrarian Research University
050010, 8 Abay Ave., Almaty, Republic of Kazakhstan
https://orcid.org/0000-0003-4145-1775
Ulzhan Nuraliyeva
PhD in Agricultural Sciences, Head of Department
Kazakh Research Institute of Livestock and Fodder Production
050035, 51 Zhandosov Str., Almaty, Republic of Kazakhstan
https://orcid.org/0000-0002-7008-7303

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Abstract. Addressing the issue of antibiotics, particularly chloramphenicol, in bee products is an urgent concern within the trade of these items due to potential risks to human health. Developing highly sensitive techniques for detecting trace amounts of antibiotic residues is crucial. This study aimed to establish an analytical method using HPLC-MS/MS (High-Performance Liquid Chromatography – Tandem Mass Spectrometry) for the identification and quantification of residual chloramphenicol

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*Corresponding author

in honey samples sourced from Kazakhstan. The developed method involved ultrasonic extraction followed by HPLC-MS/MS with electrospray ionisation in negative mode. Multiple reaction monitoring was utilised in configuring the parameters of the tandem mass spectrometric detector. The proposed procedure offered notable advantages, such as the simplicity and speed of the extraction process, as well as the sensitivity and accuracy of the detection method. The limit of detection achieved is 0.09 μg/kg, with a linear calibration curve spanning the concentration range of 0.1-1.0 μg/kg and a correlation coefficient of 0.9985. The extraction rate ranged between 97% and 101%. Importantly, this HPLC-MS/MS method for chloramphenicol determination in honey surpassed the maximum residue limit requirements (0.3 μg/kg) in terms of sensitivity, while maintaining methodological precision and a high extraction percentage. This compliance aligned with the standards outlined in Commission Decision 2002/657/EC. The method was applied to 14 honey samples from Kazakhstan, all of which were found to have chloramphenicol levels below the method's detection limit. However, when testing imported honey samples, two out of seven exceeded the detection limit for chloramphenicol. The developed HPLC-MS/MS method for detecting chloramphenicol residues in honey samples can serve as a valuable tool for routine laboratory analysis and for scrutinising potentially suspicious or questionable samples

Keywords: liquid chromatography; mass spectrometry; analysis; antibiotic; honey

INTRODUCTION

Honey is recognised as a natural product enriched with essential nutrients, including vitamins, minerals, calcium, and antioxidants, making it highly valued in both dietary and therapeutic contexts. The post-pandemic increase in consumer awareness regarding health and immunity has significantly heightened the market for natural and functional food products. The versatility of honey is seen in its extensive application within the culinary industry, including beverages, confectionary, and processed products, as well as in health, wellness, and cosmetic formulations. Its recognised therapeutic qualities, including blood pressure regulation, reduction of diabetes risk, and enhancement of wound healing, further support its commercial growth and interdisciplinary significance.

From a trade perspective, Kazakhstan exported honey valued at $490,000 in 2020, positioning the country 76th among global honey exporters (Honey in Kazakhstan, 2020). However, maintaining international competitiveness necessitates adherence to food safety regulations that prohibit the presence of specific antibiotic residues. Chloramphenicol, in particular, has been banned in food-producing animals due to its toxicological profile and association with aplastic anemia. Regulatory frameworks such as the Codex Alimentarius (CODEX STAN 12-1981), European Directives 2001/110/EC and 96/23/EC, and Commission Decision 2002/657/EC strictly regulate the permissible levels of antibiotic residues in honey (European Council, 2022).

Recent studies have drawn attention to the occurrence of veterinary drug residues in honey and the need for sensitive, reliable detection methodologies. H.O. Khalifa *et al.* (2024) emphasised the public health hazards linked to antimicrobial residues across the food chain and advocated for the adoption of enhanced detection methods, especially considering the increasing antimicrobial resistance. Y. Yang *et al.* (2022) introduced a refined QuEChERS approach integrated with UPLC-MS/MS for the detection of multi-antibiotic residues in honey, attaining elevated analytical sensitivity and confirming the system's suitability for regular monitoring of intricate matrices such as honey.

Liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) has been prominent among analytical techniques for monitoring antibiotic residues due to its exceptional selectivity, sensitivity, and compatibility with complicated matrices. Y. Zhang *et al.* (2019) evaluated LC-MS/MS techniques for the measurement of antibiotics in honey, highlighting the essential function in verifying regulatory adherence and identifying chemicals at sub-nanogram concentrations. The evaluation emphasised significant obstacles in technique standardisation and the necessity for matrix-matched calibration to guarantee accuracy in honey-based testing. The utilisation of analytical techniques to evaluate the quality and safety of honey relies on a precise comprehension of its compositional attributes (Kołacz *et al.*, 2023). A. Kobysh *et al.* (2025) illustrate the importance of investigating the botanical origin of honey, as floral and regional heterogeneity substantially affect the matrix composition and, in turn, the efficacy of residue detection methods. These findings underscore the necessity to customise analytical methods to the distinct physicochemical characteristics of honey sourced from particular ecological contexts.

E. Bonerba *et al.* (2021) examined the identification of chloramphenicol in honey samples from various production systems and emphasised the necessity for high-throughput and confirmatory procedures in standard food inspection. The research highlights the regulatory consequences of non-compliance, particularly when trace residues evade detection by less sensitive techniques. S. Saad *et al.* (2024) shown that honey contamination may arise from agricultural methods and environmental pollutants, underscoring the multifaceted nature of the issue, which includes indirect

contamination via ambient exposure. S. Abera *et al.* (2025) presented a micro-extraction-based HPLC-DAD method for multi-residue antibiotic analysis, showcasing the versatility of innovative sample preparation processes and providing an alternative to more resource-demanding solid-phase extraction methods.

Notwithstanding these developments, a significant gap exists in the literature about the creation of verified, quick, and cost-effective confirmatory procedures specifically designed for the unique physicochemical properties of honey produced in Kazakhstan. The diversity of floral sources and beekeeping practices in the region requires a method that guarantees analytical sensitivity and methodological rigour while being suitable for routine laboratory use. The purpose of this study was to develop and validate a high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method for the quantitative determination of chloramphenicol residues in honey produced in Kazakhstan, ensuring compliance with international food safety standards.

MATERIALS AND METHODS

A certified chloramphenicol (CAP) standard of 98 % purity (Sigma-Aldrich, Milan, Italy) was used for the study. The structural formula of CAP is shown in Figure 1.

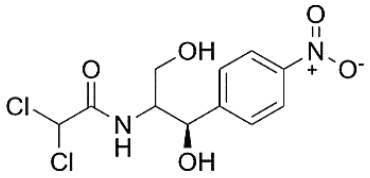

Figure 1. *Structural formula of chloramphenicol*
Source: *S. Akter Mou et al. (2021)*

Acetonitrile of HPLC grade with a purity of ⩾99.9% obtained from Sigma-Aldrich in France, acetic acid (98-100% purity) sourced from Merck in Germany, and high-purity bidistilled water produced using the Milli-Q system by Millipore Corporation were utilised in the experimental procedures. Initial CAP standard solutions were prepared at a primary concentration of 1.0 mg/ml in acetonitrile. These solutions were stored in a meticulously sealed container within a refrigerator at 4°C in a dark environment for a maximum period of two months. To create working standard solutions, successive dilutions of the primary stock solution were made with acetonitrile until a concentration of 100 mg/ml was achieved. Chromatographic analysis was conducted using the UHPLC UltiMate 3000 system from Thermo Scientific in the USA. The separation process was executed using a C18 BDS Hypersil column with dimensions of 100×4 mm. The column temperature was maintained at 35°C, and a stabilisation time of 1 minute was observed. Chromatographic separation was achieved by employing a mobile phase composed of 0.5% acetonitrile dissolved in Milli-Q water (eluent A) and 0.1% acetic acid dissolved in acetonitrile (eluent B) at a flow rate of 0.5 ml/min. The elution process commenced with 0% B and was then incrementally increased to 45% B over a span of 9 minutes. Subsequently, the elution was further increased from 45% B to 100% B over 10.5 minutes, maintained at 100% B for 1 minute, and then returned to baseline conditions after an additional 1.5 minutes. The total running time, inclusive of column equilibration to its initial state, amounted to 16 minutes. Each injection volume was set at 20 µl.

The mass spectrometric analysis was conducted using a TSQ Quantum Access MAX tandem quadrupole mass spectrometer equipped with an Ion Max™ source, employing electrospray ionisation (ESI/APCI). Negative mode electrospray ionisation was employed with a cone voltage of 40V, a desolvation temperature of 500°C, and a desolvation gas (nitrogen) flow rate of 1000 l/h. The operational parameters of the mass spectrometer were set as follows: a capillary voltage of 3.00 kV, a source temperature of 300°C, collision gas consisting of argon, and atomisation gas using nitrogen with a flow rate of 50 l/h. Data processing was accomplished utilising the Xcalibur and Chromeleon Xpress software platforms.

Honey samples were collected from various sources, including retail outlets such as markets and trade centres in Almaty, as well as from different beekeepers across diverse geographic regions within Kazakhstan. These samples were stored in a dark environment at room temperature until they underwent analysis. To construct a calibration curve, one honey sample was specifically tested for the absence of target antibiotics. For the analysis, a test sample of honey weighing 1 g was combined with 10 ml of a 0.5% acetic acid solution in Milli-Q water, and the mixture was placed in a 15 ml volumetric flask. The flask was vigorously shaken for 2-3 minutes until complete dissolution was achieved. Subsequently, the sample underwent treatment in an Elmasonic S120H ultrasonic bath, operating at 37 kHz and with ultrasonic power set at 100 W, maintaining a temperature of 50 ± 3°C for 30 minutes. After achieving complete dissolution, the sample was then subjected to centrifugation for 4 minutes at 4000 rpm. The liquid phase obtained from this process was filtered through a 0.20 µm pore size polytetrafluoroethylene membrane filter (specifically, the Macherey-Nagel Chromafil Xtra PTFE-20/25). The filtered liquid was then transferred into a vial and injected into the system using a 20 µl volume.

RESULTS AND DISCUSSION

The HPLC-MS/MS method has gained considerable appeal as an analytical technique due to its distinct features, including specificity, sensitivity, and the capability to handle multiple analytes simultaneously. These attributes have contributed to its widespread utilisation in the precise detection of minute quantities of antibiotic residues, such as chloramphenicol, as highlighted

in the work of Y. Zhang *et al*. (2019). This method is distinguished by its rapid analysis speed, superior resolution, increased peak capacity, and enhanced sensitivity.

To establish the methodology presented in this study, the precursor ion (321.1) was initially identified using the flow-through injection technique without applying collision energy. Subsequently, the optimisation of precursor ion products was carried out by introducing a standard CAP solution with a concentration of 150 mg/ml in both positive and negative polarity modes. In the analysis of the mass spectrum of chloramphenicol, it was evident that the positive ionisation mode primarily exhibited a protonated [M + H] + molecule as the principal peak, while the negative ionisation mode showed a deprotonated [M - H] - molecule. The negative ionisation mode, attributed to the deprotonation of CAP, delivered higher signal intensity and lower background noise. During the optimisation of parameters for the tandem mass spectrometric detector, particular emphasis was placed on achieving optimal conditions for generating precursor ions of CAP, including voltage at the cone and accurate m/z values based on the analyte's molecular mass. Multiple reaction monitoring (MRM) was employed to monitor the transitions from quantifier ions to qualifier ions (precursor-ion > product ion transitions, m/z). The mass spectra of standardised CAP samples prominently displayed deprotonated molecular signals at m/z 321.1. Additionally, three distinctive product ion fragmentations (151.9, 193.6, and 256.8) were obtained and monitored using appropriate collision energy settings (see Table 1 for details).

Table 1. *Multiple reaction monitoring method (MRM method) for CAP determination*

Precursor ion, m/z	Product ion, m/z	Taper voltage, V	Collision energy, V	Holding time, min
321.1	151.9	40	18	7.09
	193.6		12	
	256.8		11	

Source: *compiled by the authors based on H. Ye et al. (2022)*

The product ion mass spectra prominently featured ions with an m/z value of 151.9 (as depicted in Fig. 2), clearly corresponding to the loss of a C2H3OH-NHCOCH-Cl2 group. Additionally, other ion products observed in the MS/MS spectra included m/z 193.6 (indicative of the loss of NH2COCHCl2) and m/z 256.7 (associated with the loss of HCl and CO). In the quantification of CAP, it was considered three product ions, aligning with the requirements stipulated in Commission Decision 2002/657/EC, which implements Council Directive 96/23/EC regarding the performance of analytical methods and the interpretation of results (2002). The separation process was conducted by passing the sample through a chromatographic column, with the predominant product ion (m/z 151.9) exhibiting a retention time of 7.08 minutes (as illustrated in Fig. 3).

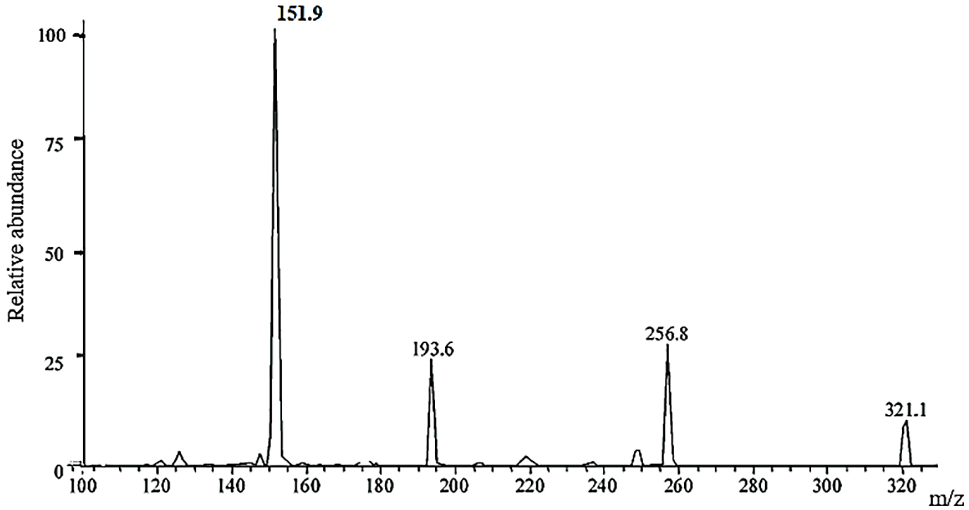

Figure 2. *Mass spectra of chloramphenicol product ions*
Source: *compiled by the authors based on B. Vuran et al. (2021)*

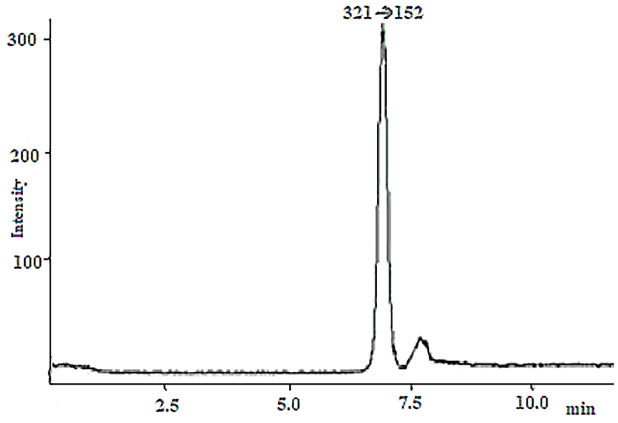

***Figure 3**. MRM chromatogram of chloramphenicol*
***Source:** compiled by the authors based on S. Rizzo et al. (2020)*

Effective sample preparation plays a crucial role in the overall analytical process. Given the intricate composition of honey samples and the stringent maximum residue limit (MRL) set for CAP in honey (MRL = 0.3 μg/kg), it is imperative to employ a robust sample processing technique that eliminates matrix interferences and concentrates CAP before its quantification, as emphasised by R. Pratiwi *et al.* (2023). The intricate composition of the honeycomb matrix itself, enriched with substantial quantities of sugars, enzymes, and proteins, demands a specialised approach to sample preparation. Notably, effective deposition of these components is essential because soluble proteins present in the sample extract can introduce significant matrix effects, disrupt phase separation during organic solvent extraction and sample purification, distort the analytical signal, and exert an adverse impact on the integrity of the chromatographic column. Consequently, this can lead to an overall increase in pressure within the chromatographic system, as highlighted by H.-N. Jung *et al.* (2022). To overcome these challenges, diverse methods are employed to optimise the sample preparation protocol, with the primary goal of achieving maximal extraction efficiency and recovery rates.

Among the widely adopted techniques, liquid-liquid extraction (LLE) and solid-phase extraction (SPE) stand out as the most prevalent (Yakubchak *et al.*, 2018). However, it's worth noting that LLE involves the use of solvents such as ethyl acetate, acetonitrile, chloroform, and hexane. In contemporary trends within analytical chemistry, there is a concerted effort not only to streamline sample preparation methods but also to minimise the reliance on organic solvents (Sniegocki *et al.*, 2019; Ilderbayeva *et al.*, 2024). The composition of the adsorbent can be considered as the basis of the SPE method, as it influences the selectivity and the absorption capacity. The most commonly used Oasis HLB and Strata-X cartridges offer low matrix effect and high extraction rates. However, a common disadvantage of the SPE method is that such a procedure requires additional resources and time, which appreciably increases the cost of analysis. The fast, simple, cheap, efficient, reliable, and safe (QuEChERS) method also finds application as compared to the time-consuming and expensive SPE, QuEChERS can be very flexibly modified into several forms by changing the type and amount of sorbents (Vuran *et al.*, 2021). However, the QuEChERS procedure for honey typically involves extraction, protein precipitation and purification (Veiga-del-Baño *et al.*, 2024). After extracting the solid sample using acetonitrile in an aqueous medium, a subsequent step involves a liquid-solid extraction, often referred to as dispersion solid-phase extraction (SPE). This phase aims to eliminate the majority of residual matrix interferences, albeit introducing some complexity to the analysis process.

To establish a confirmatory method for detecting chloramphenicol residues in honey sourced from Kazakhstan, authors adopted the approach described in section 2, which involves ultrasonic extraction using an aqueous acetic acid solution followed by input into an HPLC system without additional purification steps. While literature reports on the application of ultrasonic extraction have primarily focused on extracting phenols from olive oil (Kivrak & Kivrak, 2021), polyphenols (Rivera-Mondragón *et al.*, 2019), phenolic acids from plants used in traditional medicine (Sukor *et al.*, 2018; Garibli *et al.*, 2021), and mycotoxins from cereal products (Streit *et al.*, 2022), there is a notable scarcity of studies regarding the development of an ultrasonic method for chloramphenicol determination in honey using high-performance liquid chromatography and tandem mass spectrometry. In the formulation of the method employed in this study, the selection of acetic acid as the extraction solvent and the parameters for ultrasonic treatment were determined through preliminary experiments and a thorough analysis of limited literature data (Manimekalai *et al.*, 2019). Since cost-effectiveness is one of the critical characteristics of a technique designed for routine analysis of food of animal origin for quality and safety parameters, the advantage of optimised sample preparation is that in this system a purification stage, solid-phase extraction is not necessary. The uniform distribution of ultrasound in the liquid allows the mixture to be mixed and homogenised for ultrasonic extraction.

The CAP content of the tested honey samples was carried out according to a calibration curve using Xcalibur and Chromeleon Xpress software. The calibration curve was plotted against a matrix of pure (control) honey in the concentration range of 0 to 1.0 μg/kg, and was linear in the interval studied (Fig. 4). The correlation coefficient was 0.9985. The validation of the developed method for the analysis of honey samples was carried out by testing a control (pure) honey sample and one loaded with a standard solution at four different

concentration levels with concentration calculations from the calibration graph on the honey matrix. Samples were analysed for three consecutive days to determine precision and validity. The extraction rate was 97-101%, which meets the requirement of 50 to 120% (Commission decision..., 2002).

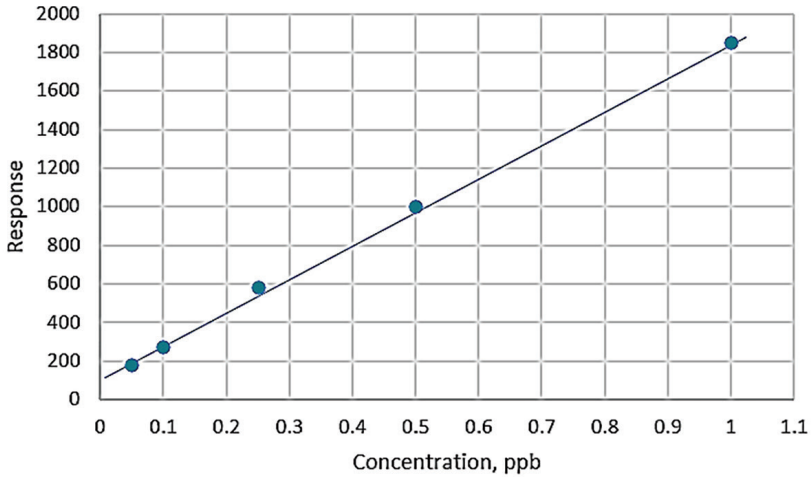

***Figure 4**. Calibration chart for the determination of chloramphenicol in honey*
***Source:** compiled by the authors based on M. Rydchuk et al. (2019)*

The limit of detection (LOD) and limit of quantification (LOQ) values were assessed and found to be 0.09 μg/kg and 0.18 μg/kg, respectively. In the context of honey quality control, various countries have established maximum residue limits (MRL) for chloramphenicol in honey, such as 0.3 μg/kg in European Union nations, 0.1 μg/kg in Belgium, and a complete absence of chloramphenicol allowed in the USA. From the data presented, it's evident that the developed HPLC-MS/MS method for chloramphenicol determination in honey surpasses the sensitivity requirements set by the established MRL of 0.3 μg/kg. This achievement is coupled with a commendable level of methodological precision and a high percentage of extraction, thus aligning with the stipulations outlined in Commission Decision 2002/657/EC, which implements Council Directive 96/23/EC concerning the performance of analytical methods and result interpretation (2002), particularly with regard to confirmatory methods.

The developed HPLC-MS/MS methodology for determination of chloramphenicol in honey was tested in the analysis of 7 samples of honey produced in Kazakhstan, taken in different retail outlets (markets, trade centre) in Almaty and 7 samples taken from several beekeepers from different geographical regions of Kazakhstan. None of the samples tested contained the target analyte at a level above the detection limit of the developed method. Honey contamination can be linked to intensive farming and industrial activities. In addition, beekeepers are not always registered as defined by legislation, can purchase veterinary medicines themselves (without prescriptions) and do not keep records on the treatment of bees (indicating the date of application, name of the medicine and period of excretion). The presence of chloramphenicol residues in honey can also be attributed to the use of products containing a combination of chloramphenicol and its SS-pair stereoisomer, dextramycin. It's important to note that dextramycin lacks therapeutic efficacy; nevertheless, it has been frequently detected in unreported quantities within counterfeit bee preparations, leading to its inadvertent presence in honey (Yanovych *et al.*, 2018). The enzyme immunoassay method, most commonly used for incoming inspection of raw materials in honey processing plants, does not provide adequate control for dextramycin residues (Dvykaliuk *et al.*, 2023).

In addition to the "human factor" that directly causes contamination of bee products, there are also indirect factors. For example, the use of Styrofoam or technical foils with foil to insulate the top of the nest can give false positives for the presence of chloramphenicol. Furthermore, it's important to acknowledge that the dataset under analysis may not be considered highly representative due to its reliance on a limited number of honey samples acquired through random sampling. Nevertheless, the results obtained unmistakably demonstrate the efficacy of this method for detecting CAP residues in routine analysis, making it suitable for adoption by production and quality control laboratories.

The established HPLC-MS/MS technique for quantifying CAP in honey samples from Kazakhstan has exceptional sensitivity, methodological accuracy, and economical sample preparation. An analysis alongside comparable academic studies highlights both similarities and differences that emphasise the originality and practical importance of the current study. G. Cilia *et al.* (2020) examined the antibacterial properties of Ukrainian honey, uncovering considerable regional

heterogeneity in bioactivity, which they ascribed to floral origin. While the work did not explicitly evaluate synthetic antibiotic residues, the focus on compositional variability corresponds with the methodological approach to matrix calibration and underscores the necessity of customising detection methodologies to the physicochemical characteristics of locally produced honey. S.A. Dar *et al.* (2024) provided a comprehensive analysis of honey classification, composition, and contamination hazards, specifically concerning veterinary drug residues. The observations concerning the frequency of such residues in emerging markets underscore the significance of the study, which addresses this issue with a validated HPLC-MS/MS method capable of measuring chloramphenicol at levels well below internationally approved MRLs.

M. Hutchings *et al.* (2019) offered a historical perspective on antibiotic development and expressed apprehensions regarding the enduring residues in the food chain that contribute to antimicrobial resistance. The following research directly tackles this issue by introducing a swift and sensitive confirmatory approach to avert low-level residues of prohibited antibiotics, such as chloramphenicol, from contaminating the food supply, thereby providing a preventive framework against the hazards identified by M. Hutchings *et al.* A more detailed methodological comparison is apparent with Y. Li *et al.* (2021), who created a quantum dot-based immunochromatographic strip for the swift detection of antibiotics in honey. While the methodology enabled on-site testing and offered multiplex functionality, it was deficient in confirmatory robustness and precision compared to tandem mass spectrometry. Conversely, HPLC-MS/MS technology, although more labour-intensive, provides superior analytical precision and adheres to regulatory criteria, including Commission Decision 2002/657/EC (2002).

C.M.G. Lima *et al.* (2020) performed a comprehensive assessment of antibiotic residues in honey and emphasised the related public health issues. The research promoted ongoing surveillance and enhanced testing techniques, underscoring the significance of the method's relevance to national residue monitoring initiatives in Kazakhstan. Consistent with the findings, this study indicated the possible presence of CAP in imported honey samples, emphasising the need for rigorous import regulations. R. Phipps (2025), in his assessment on the international honey market, highlighted the growing regulatory oversight in global honey trading, particularly inside the EU. The findings of the following study affirm that sophisticated analytical techniques are essential for maintaining the export competitiveness of Kazakh honey, especially in areas where regulatory adherence is strongly linked to trade access.

G.G. Rimkus *et al.* (2020) underscored the constraints of immunoassays, such as ELISA, in identifying stereoisomeric variants of chloramphenicol, including dextramycin, potentially resulting in false-negative outcomes. The mass spectrometric technique surmounts this constraint by precise ion fragmentation and MRM-based detection, facilitating accurate separation and quantification of chloramphenicol, thus enhancing reliability. S. Rizzo *et al.* (2020) devised a salting-out aided liquid-liquid extraction (SALLE) method integrated with LC-MS/MS for the detection of CAP. The salting-out method, however effective, necessitates more complex sample preparation. Conversely, the present study's ultrasonic extraction utilising aqueous acetic acid provides a more efficient, environmentally sustainable, and expedited alternative, without sacrificing extraction efficacy or method validation.

M. Rydchuk *et al.* (2019) developed a UPLC-MS/MS multimethod for the detection of diverse antimicrobial substances in honey, emphasising the capability for concurrent analysis. Although the approach exhibited remarkable sensitivity, this study is unique in its specific targeting of chloramphenicol and in refining a more straightforward ultrasonic extraction protocol devoid of supplementary purification steps, thereby enhancing its practicality for routine domestic testing laboratories with constrained resources. These comparisons confirm that although previous studies have greatly enhanced antibiotic residue detection in honey, the current study progresses the field by providing a validated, resource-efficient, and regulation-compliant analytical method specifically designed for the unique physicochemical properties of Kazakh honey. The results not only address current methodological deficiencies but also offer a reproducible framework for national quality control systems.

CONCLUSIONS

A precise, dependable, rapid, and highly sensitive technique has been established for the detection of chloramphenicol in honey sourced from Kazakhstan. This method leverages a high-performance liquid chromatograph coupled with a tandem quadrupole mass spectrometric detector employing negative mode electrospray ionisation. In setting the parameters of the tandem mass spectrometric detector special attention was paid to establishing the optimum conditions for obtaining chloramphenicol precursor ions – cone voltage and accurate m/z value. Multiple reaction monitoring was used to track precursor-ion > product-ion transitions (m/z). As a result of optimised HPLC-MS/MS parameters, high sensitivity, and selectivity of target component extraction were observed. The analytical performance of the developed methodology was improved with almost no additional time and resource consumption during the sample preparation step. The analyte was extracted using ultrasound and introduced into the HPLC system without the use of additional purification methods such as solid-phase extraction. The method adhered to the confirmatory standards outlined in Commission

Decision 2002/657/EC, which implements Council Directive 96/23/EC governing the execution of analytical techniques and the interpretation of the outcomes.

14 samples of honey from Kazakhstan were analysed using the developed method. It was found, that none of the samples contained the target analyte at a level above the detection limit of the method. When analysing samples of imported honey, in two samples out of 7 the amount of CAP exceeded the permissible norms. The application of the indicated method in laboratories would limit the uncontrolled use of antibiotics in beekeeping, ensure high quality of honey and hence increase its competitiveness. This method holds valuable potential for monitoring and controlling the illicit utilisation of CAP in food production. It represents a crucial step in assessing the ramifications of unauthorised CAP use, whether by beekeepers employing prohibited preparations or medicines readily accessible in retail pharmacies intended for human use. Furthermore, this methodology could serve as a foundational framework for the creation of an HPLC-MS/MS technique capable of detecting and quantifying CAP, and potentially other antibiotic residues sharing structural similarities, across various food matrices. New research questions and perspectives emerged in the course of the study. The study of other analytes used in beekeeping whose residues in honey can be determined by the developed HPLC-MS/MS methodology, without significant modification of the sample preparation procedure and chromatographic system parameters. Exploring additional bee-derived products like beeswax, bee venom, pollen, and royal jelly is a promising avenue. These substances find application both as dietary supplements and in medicinal contexts, making them worthy subjects of investigation.

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CONFLICT OF INTEREST

None.

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Розробка методу ВЕРХ-МС/МС для кількісного визначення хлорамфеніколу в меді з Казахстану

Фаріда Бактибаєва
Магістр технічних і біологічних наук, докторант
Казахський національний аграрний дослідницький університет
050035, вул. Жандосова, 51, м. Алмати, Республіка Казахстан
https://orcid.org/0000-0002-6279-2731
Асія Серікбаєва
Доктор біологічних наук, професор
Казахський національний аграрний дослідницький університет
050035, вул. Жандосова, 51, м. Алмати, Республіка Казахстан
https://orcid.org/0009-0005-8659-9230
Максат Тойшиманов
Магістр технічних наук, інженер
Казахський національний аграрний дослідницький університет
050035, вул. Жандосова, 51, м. Алмати, Республіка Казахстан
https://orcid.org/0000-0002-6070-4574
Максат Серіков
Магістр технічних наук, науковий співробітник
Казахський національний аграрний дослідницький університет
050035, вул. Жандосова, 51, м. Алмати, Республіка Казахстан
https://orcid.org/0000-0003-4145-1775
Улжан Нуралієва
Кандидат сільськогосподарських наук, завідувач кафедри
Казахський національний аграрний дослідницький університет
050035, вул. Жандосова, 51, м. Алмати, Республіка Казахстан
https://orcid.org/0000-0002-7008-7303

Анотація. Проблема наявності антибіотиків, зокрема хлорамфеніколу, у продуктах бджільництва є актуальною у зв'язку з ризиками для здоров'я людини та впливом на торгівлю цими продуктами. Важливим завданням є розробка високочутливих методів для виявлення залишкових кількостей антибіотиків. Метою цього дослідження було створення аналітичного методу із застосуванням ВЕРХ-МС/МС (високоефективна рідинна хроматографія – тандемна мас-спектрометрія) для ідентифікації та кількісного визначення залишків хлорамфеніколу в зразках меду з Казахстану. Запропонований метод передбачав ультразвукову екстракцію з подальшим аналізом за допомогою ВЕРХ-МС/МС з електроспрейною іонізацією в негативному режимі. Параметри тандемного мас-спектрометричного детектора налаштовано з використанням режиму множинного моніторингу реакцій. Запропонована методика мала значні переваги, зокрема простоту та швидкість екстракції, а також високу чутливість і точність виявлення. Межа виявлення становила 0.09 мкг/кг, а калібрувальна крива була лінійною в діапазоні концентрацій 0.1-1.0 мкг/кг з коефіцієнтом кореляції 0.9985. Вихід екстракції становив від 97 % до 101 %. Важливо зазначити, що цей метод визначення хлорамфеніколу за допомогою ВЕРХ-МС/МС перевищував вимоги до граничного рівня залишків (0.3 мкг/кг) за чутливістю, водночас зберігаючи високу точність та ефективність екстракції. Метод відповідав вимогам, викладеним у Рішенні Комісії 2002/657/ЕС. Метод було апробовано на 14 зразках меду з Казахстану, в жодному з яких не було виявлено хлорамфеніколу вище межі виявлення. Водночас у двох із семи зразків імпортного меду концентрація хлорамфеніколу перевищувала межу виявлення. Розроблений метод ВЕРХ-МС/МС може бути ефективним інструментом для рутинного лабораторного аналізу та перевірки підозрілих або сумнівних зразків меду

Ключові слова: рідинна хроматографія; мас-спектрометрія; аналіз; антибіотик; мед