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A modified quick, easy, cheap, effective, rugged, and safe cleanup method followed by liquid chromatography-tandem mass spectrometry for the rapid analysis of perchlorate, bromate and hypophosphite in flour

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ABSTRACT

A selective, sensitive and useful method, based on modified QuEChERS cleanup combined with liquid chromatography-tandem mass spectrometry (LC–MS/MS) in the negative-ion electrospray ionization (ESI^-) mode, was developed and validated for the simultaneous determination of three inorganic anions including perchlorate (ClO_4^-), bromate (BrO_3^-) and hypophosphite ($H_2PO_2^-$) in flour. The extraction parameters and LC–MS/MS conditions were optimized by single-factor experiment and sorbent combination in modified QuEChERS clean-up was optimized through response surface analysis. Three target analytes were separated on a normal-phase Phenomenex Luna Silica (2) column (150 mm × 2.0 mm, 5 μm, 100 Å) with the mobile phase of a mixture of 5 mmol/L ammonium acetate water solution and acetonitrile, detected by MS/MS under multiple reaction monitoring and quantified by external standard method. The developed method was validated in terms of the sensitivity, linearity, accuracy and precision, and matrix effect. The method showed a good linearity ($R^2 > 0.999$) for all analytes in their respective concentration ranges. The ILOQs and MLOQs for perchlorate, bromate and hypophosphite were 0.1, 0.5, 5.0 μg/L and 2.0, 6.0, 60.0 μg/L, respectively. The average recoveries of three target analytes from the negative samples spiked at three different concentrations were in a range from 84.6% to 104.9%. The intra-day precision ($n = 6$) and inter-day precision ($n = 5$) of the target analytes were in the ranges of 2.9%–6.9% and 6.4%–8.2%. The matrix effect of this method was observed between 0.83 and 1.17 and was acceptable. The validated method was successfully applied to determine the concentrations of these inorganic anions in flour. Results found that perchlorate and hypophosphite were detected in 33 out of 50 and 7 out of 50 flour samples.

1. Introduction

Perchlorate anion (ClO_4^-) is an environmental contaminant which is persistent in the environment due to its high solubility and extreme stability in aqueous media. It is easily transferred to the ground and surface water and also appears to soil and food [1,2]. Perchlorate contamination in drinking water and even in food products has recently caused the extensive concern due to its possible adverse impact on human health at relatively low concentrations [3–5]. It has been reported that high concentrations of perchlorate could interfere with iodide uptake and block its absorption in the thyroid. The resulting malfunction of the Na^+-I^- symporter could reduce thyroid hormone production and pregnant women, children, and people with compromised thyroid functions are particularly at risk [5–7]. Perchlorate has been detected ubiquitously in vegetables such as lettuce [8,9], fruits such as cantaloupe [10], bottled water [11], milk [12] and a variety of other edible foods [1,13,14].

Bromate (BrO_3^-) has been widely used in flour products to enhance their strength and even the "whiteness", which results in consumer-appealing properties such as make the flour stronger and more extensible [15]. Nevertheless, bromate is considered to be one of the emerging water contaminants and has been reported to be harmful to human kidney. It has been classified as a Group 2 B carcinogen by the International Agency for Research on Can-

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cer (IRAC) and the World Health Organization has established a lifetime cancer risk of 1 in 10^5 for 3 μg/L for bromate. Therefore, IRAC suggested that bromate should not be present in food [16,17]. Unfortunately, bromate is still misused in flour and related products due to its low price and favorable property. Hypophosphite ($H_2PO_2^-$) can also be used in flour products to improve the gluten intensity. In our routine investigation, we found that hypophosphite was added in flour and flour improver by several dishonest manufacturers. However, although very little is known about the harm effects to human health, hypophosphite has not been listed as a kind of food additive in GB 2760-2014 of China National Standard.

Wheat flour, which can be used to make many daily foods such as bread, noodles, dumplings, etc, is considered to be one of the most widely consumed food materials in China [15]. However, flour is easily contaminated with ClO_4^-, BrO_3^- and $H_2PO_2^-$ by deliberately artificial addition or spontaneous introduction from environment [1–3,6–8,15,16]. Therefore, the quality and security of flour and related foods are very important for our health and the development of sensitive and robust methods for these inorganic anions analysis in flour is of great importance.

Analytical methods developed recently for the determination of ClO_4^- and BrO_3^- mainly include ion chromatography (IC) [5,6,15], ion chromatography with inductively coupled plasma mass spectrometric detection (IC-ICP/MS) [18], and ion chromatography tandem triple quadrupole mass spectrometry (IC-MS/MS) [19,20]. As for $H_2PO_2^-$ determination, the most commonly used method is the chemical titration method. However, IC is only suitable for the determination of simple matrix such as water, while it is vulnerable to be interfered by matrix in complex matrix analysis, resulting in inaccurate quantification. With respect to IC-MS/MS, strong alkaline solution used as the mobile phase in IC is easy to contaminate the MS, resulting in inaccurate determination and life time decrease of MS. Chemical titration method is not applicable for the determination of complex food matrix or low concentrations of target analytes.

In recent years, LC–MS/MS is widely used in the analysis of complex matrix due to its excellent selectivity and anti-interference capability [21,22]. Some researchers have adopted LC–MS/MS in the determination of ClO_4^- and favorable results were obtained [12,13,23]. For example, in a previous literature, a reversed-phase LC–MS/MS method was developed for the analysis of perchlorate in water and the developed method was confirmed to be rapid, accurate, and reproducible with the LOD of 0.4 μg/kg [12]. Determination of perchlorate in soil matrix by LC–MS/MS was also developed and acceptable recoveries and LOD of 0.5 μg/kg for soil sample were obtained [23]. Nevertheless, the monotonous detection of target analytes restricted its wide application.

QuEChERS cleanup, also known as dispersive solid phase extraction (dSPE), has firstly been developed for analyzing the pesticide residues in vegetables and fruits [24]. The solid sorbent used in this technique is added directly to the sample solution without processes of sample manipulation such as conditioning, so the cleanup procedure relies only on shaking and centrifugation [25]. QuEChERS cleanup has draw more and more attention due to its quick, easy, cheap, effective, rugged and safe advantages and has been widely used in the analysis of complex food matrix [21,26,27].

Therefore, the aim of the present study was to develop a selective, sensitive and modern modified QuEChERS cleanup combined with LC–MS/MS method for the simultaneous determination of perchlorate (ClO_4^-), bromate (BrO_3^-) and hypophosphite ($H_2PO_2^-$) in flour. The pretreatment extraction, QuEChERS cleanup and instrumentation parameters were optimized through single-factor experiment and response surface analysis. Then, the developed method was validated in terms of linearity, selectivity, accuracy and precision. Finally, the validated method was applied to analyze 50 flour samples from different commercial markets and producers.

2. Materials and methods

2.1. Regents and standards

Perchlorate standard solution (ClO_4^-, CAS No.: 7601-89-0, purity: 100%, 1000 mg/L), sodium hypophosphite monohydrate ($NaH_2PO_2{\cdot}H_2O$, CAS No.: 10039-56-2, purity: 97%) and potassium bromate ($KBrO_3$, CAS No.: 7758-01-2, purity >99.8%) were purchased from Sigma-Aldrich (St. Louis, USA). Acetonitrile (HPLC grade) was obtained from Fisher Scientific (Fair Lawn, USA). Ammonium acetate (HPLC grade) was purchased from Tedia (Weston, USA). Three kinds of sorbents including graphitized carbon black (GCB), octadecylsilane (C18) and primary secondary amine (PSA, 40–63 mm, 6 nm) were supplied by CNW Technologies GmbH (Düsseldorf, Germany). Water was purified with a Milli-Q system (Millipore, Bedford, MA, USA) to obtain the ultrapure water (18.2 MΩ·cm) which was used in the whole experiment.

Individual standard stock solutions (1000 mg/L) of bromate (BrO_3^-) and hypophosphite ($H_2PO_2^-$) were prepared by dissolving potassium bromate and sodium hypophosphite monohydrate in water, respectively. The mixed standard solution was prepared by diluting each individual standard stock solution with 50% acetonitrile water (*v*/*v*) to the concentrations of 0.5 mg/L, 2.5 mg/L and 25 mg/L for ClO_4^-, BrO_3^- and $H_2PO_2^-$, respectively. The above individual standard stock solutions and the mixed standard solution were subsequently stored in a refrigerator at 4 °C. The required mixed standard working solutions were obtained by further dilution of the mixed standard solution with 50% acetonitrile water (*v*/*v*) prior to use.

2.2. Sample preparation

Approximately, 1.0 g of flour was accurately weighed into a clean 50 mL plastic centrifuge tube. Then, 10 mL of 50% acetonitrile water (*v*/*v*) was added to dissolve the flour and this solution was vortexed by a MS3 basic vortex mixer (IKA GmbH, Germany) for 2 min. The homogenized solution was then sonicated in a KQ-250DV ultrasonic bath (Kunshan, China) for 10 min and centrifuged with a 5418 high speed centrifuge (Eppendorf Corp., Germany) at 8 000 r/min for 5 min. After that, 1 mL of the supernatant was added into a 2 mL centrifuge tube which contains 100 mg of C18 sorbent and 50 mg of GCB sorbent. It was vortexed for 1 min and centrifuged at 12 000 r/min for 5 min. Finally, the supernatant was prepared for LC–MS/MS analysis.

2.3. LC–MS/MS conditions

The LC–MS/MS system consists of a Waters ACQUITY™ UPLC system connected to a Waters Xevo™ TQ tandem triple quadrupole mass spectrometer (Waters Corp., Beverly, MA). LC analysis was performed on three different columns including two reversed-phase columns (Waters BEH C18 column (100 mm × 2.1 mm, 1.7 μm), Diamonsil C18(2) column (150 mm × 4.6 mm, 5 μm)) and a normal-phase column (Phenomenex Luna Silica (2) column (150 mm × 2.0 mm, 5 μm, 100 Å)) at a flow rate of 0.4 mL/min. The column temperature was set at 30 °C and the peak shapes, resolutions and separation efficiencies of target analytes on these columns were compared. The mobile phases were 5 mmol/L ammonium acetate water solution (A) and acetonitrile (B) and three inorganic anions were eluted with a gradient elution program. The gradient for the finally selected column (Phenomenex Luna Silica (2) column) was programmed as follows: 0–3.0 min, 70% B; 3.0–4.0 min, 70%–30% B; 4.0–5.0 min, 30%–20% B; 5.0–5.1 min, 20%-70% B; 5.1–8.0 min, 70% B. The injection volume was 5 μL. In order to avoid the contamination of mass spectrometer, the chromato-