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| एफएसएसएआई fssai भारतीय खाद्य सुरक्षा और मानक प्राधिकरण Food Safety and Standards Authority of India स्वास्थ्य और परिवार कल्याण मंत्रालय Ministry of Health and Family Welfare | **Residue Analysis of Ethylene Oxide and 2-Chloroethanol in Foods by Gas Chromatography Tandem Mass Spectrometry** | | |
| **Method No.** | FSSAI.OM.ETO.001.2023 | **Revision No. & Date** | 0.0 |
| **Introduction/ Caution** | This is a selective and sensitive method for the residue analysis of ethylene oxide (EO) in diverse matrices, namely oilseeds, cereals, tea, spices, herbs, dehydrated fruits, food additives, and fruits & vegetables. When a food item is treated with EO (as a fumigant) to control spoilage and public health related microorganisms, it rapidly reacts with the matrix components, especially chloride, to form 2- chloroethanol (2-CE).<br><br>EO is reportedly carcinogenic (e.g., lymphoma and leukemia), mutagenic, and reprotoxic, and is not approved as per the Food Safety and Standards Act. Similarly, 2-CE demonstrates carcinogenic and reproductive toxic properties in some studies.<br><br>Here, a modified QuEChERS (EN 15662) technique is documented for the rapid analysis of EO and 2-CE using (PTV)-GC-MS/MS or HS-GC-MS/MS. When the method is validated in a wide variety of dry and fresh (high-moisture) food matrices, the measurement accuracies and precisions are noted in accordance with the analytical quality control criteria. | | |
| **Principle** | EO is highly volatile, and hence it is important to prepare the samples at a temperature of < 10 °C. It is possible to estimate both compounds by liquid as well as headspace GC injections. It is also possible to analyze these compounds by automated headspace (HS)-trap GC-MS, in which, syringe-based HS is combined with cryogen-free trapping technology, exploiting the multi-step sample enrichment capability to increase the method sensitivity.<br><br>EO and 2-CE are analyzed within the same GC-MS/MS run. The recovery and precision, when checked through intra- and inter-laboratory validation studies, are highly satisfactory. | | |
| **Apparatus** | A weighing balance with high precision (Vibra, Adair Dutt, Mumbai, India) is used to weigh the certified reference standards of EO and 2-CE. A heavy-duty grinder is used for crushing the samples. A vortex mixer (shaker), a highspeed refrigerated centrifuge, and a microcentrifuge are used at different stages of sample preparation. | | |
| **Chemicals** | (a) Chemicals — EO (1000 µg/mL, in methanol) and 2-CE (100 µg/mL, in methanol) having a purity >98%. HPLC grade water, anhydrous magnesium sulfate, sodium chloride, trisodium citrate dihydrate, and disodium citrate sesquihydrate. PSA: Bondesil, 40 µm particle size) and octadecylsilane (C18, ODS).<br>(b) Materials— Polytetrafluoroethylene (PTFE) syringe filters (0.22 µm) and Ultipor Nylon-6,6 membrane filters (0.2 µm pore size and 13 mm diameter) | | |

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| **Preparation of standards and reagents** | **(a) Solutions—**<br>1. Due to the high volatility of EO, its standard solutions were prepared at a low temperature < 10 °C) using a thermocol box containing ice bags.<br>2. As a diluting solvent, acetonitrile was placed in a freezer for at least 15 min before use.<br>3. The cold analytical standard solutions were pipetted into acetonitrile to generate the working standard solutions of EO (1 mg/mL) and 2-CE (1 mg/mL).<br>4. By serial dilution in acetonitrile, the calibration standards of 2.5, 5, 10, 25, and 50 µg/L were prepared from this working standard.<br>5. The matrix-matched standards of the same concentrations were also separately prepared.<br>6. Prior to extraction, all stock solutions were preserved at a temperature of −20 °C to avoid degradation losses. |
| **Preparation of Test Samples** | (a) Sample type — The samples of dry commodities [e.g., oil seeds (sesame seed), cereal (wheat), spices (cumin seed, turmeric powder, chilli powder, ginger powder), pulses (moong bean), dehydrated fruits/vegetables (kiwi, mango, onion flakes), medicinal herbal powder (e.g., ashwagandha, *Withania somnifera*), black tea powder], high-moisture foods (e.g., tomatoes, grapes), food additives (e.g., guar gum, locust bean gum), processed spices (e.g. coriander powder, curry powder mix (mutton, egg, and vegetable flavors) were evaluated for method performance. Before analysis, the samples were placed in separate sample collection bags, transferred into airtight containers, and maintained at −20 °C until further use.<br>(b) Sample preparation — With the modified QuEChERS (EN 15662) technique, the extraction of all dry and high-moisture matrices was performed at a temperature of ≤10 °C. The well-homogenized and pre-cooled samples (4 g of powdered dry matrices and 10 g of high moisture matrices) were separately taken in 50 mL polypropylene centrifuge tubes. In the case of dry matrices, 5 mL of ice-cold water was added, and the sample was left standing for 15−20 min before vortexing for 2 min. To it, 10 mL of pre-cooled acetonitrile was added for extraction and vortexed for 15 min. With the exception of the addition of water, the same procedure was followed for the high moisture matrices, e.g., grape, tomato, etc. The mixture was vortexed for 2 min with $MgSO_4$ (4 g), NaCl (1 g), trisodium citrate dihydrate (1 g), and disodium citrate sesquihydrate (500 mg). Thereafter, the mixture was centrifuged for 5 min at 5000 rpm at ≤10 °C. For the dry matrices, an aliquot of 1 mL of the cleaned supernatant was drawn and vortexed with 25 mg PSA + 25 mg C18 + 150 mg anhydrous $MgSO_4$. But for certain other (relatively complex) dry matrices, such as coriander powder, curry powder mix (mutton, egg, and vegetable flavor), ashwagandha powder, guar gum, and locust bean gum, an aliquot of 1 mL of the cleaned supernatant was drawn and vortexed with 50 mg PSA + 50 mg C18 + 150 mg anhydrous $MgSO_4$.<br>For high moisture matrices, grapes and tomatoes, 50 mg of PSA and 150 mg of anhydrous $MgSO_4$ were added and vortexed for 30 s, followed by centrifugation at 10000 rpm for 5 min (≤10 °C). |

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<tr><td></td><td>The extracts were analyzed by GC-MS/MS after filtration. A PTFE syringe filter (0.22 µm) was used for filtration of dry matrices, and a Nylon-6,6 membrane filter (0.2 µm) was used for the filtration of wet matrices.<br><br>In multi-step enrichment HS-trap, the samples are incubated at 70 ºC for 10 min with agitation at 300 rpm. To concentrate the analytes, three headspace volumes (5 mL each) are collected from each sample and injected to a focusing trap, which is electrically cooled to -30 ºC throughout the enrichment process. An incubation period of 3 min in between each extraction re-establishes the headspace equilibrium. Finally, the trap is desorbed at 250 ºC to transfer the analytes to the GC–MS system for separation and detection.</td></tr>
<tr><td>Chromatography conditions</td><td>GC- MS/MS analysis by liquid injection—<br>1. A triple quadrupole GC-MS/MS with autosampler.<br>2. The GC separation was achieved on a Wax column (e.g., TG-Wax, 30 m × 0.25 mm, 0.25 µm film thickness).<br>3. Ultrapure helium (99.9999%) was used as the carrier gas, with a flow rate of 1.2 mL/min.<br>4. The oven temperature program to be set as follows: an initial temperature of 45 °C (2 min hold) was ramped to 230 °C at 50 °C/min (5.3 min hold), which resulted in a total run time of 11 min.<br>5. The transfer line and ion source temperatures were maintained at 250 and 230 °C, respectively. The PTV injector program started at 90 °C (0.8 min hold) and increased to 250 °C (10 min hold) by rapid heating at 12 °C/s.<br>6. Split injection was employed with a gold-baffled PTV injector linear (2, 2.75, and 120 mm). The injection volume was 2 µL.<br>7. The SRM transitions may be optimized for EO and 2-CE by using the auto-SRM feature of the software.<br>8. The SRM method was automatically optimized in terms of the precursor ions, product ions, and collision energies by adjusting the dwell time for each transition to achieve the highest sensitivity (S/N).<br><br>GC- MS/MS analysis by headspace injection— A gas chromatograph with auto-injector and headspace sampler is used with a triple quadrupole mass spectrometer. The software is used for data analysis and quantitation. The hardware system allows the sample vial to enter the oven from the bottom, reducing heat loss during the process. Through an advanced flow control system, the accuracy of flow rate is maintained. The pressure in the vial is kept constant at 160 kPa.<br><br>Dynamic headspace GC-MS/MS parameters for EtO and 2-CE:<br><table><tr><td>Column</td><td>Rtx-VMS GC column: 60 m × 0.45 mm, 2.55 µm or equivalent</td></tr><tr><td>Flow rate</td><td>Helium, 3.0 mL/min</td></tr></table></td></tr>
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<tr><td>Injection mode</td><td colspan="4">Split, split ratio: 20:1</td></tr>
<tr><td>Headspace program</td><td colspan="4">Incubation temperature: 110 °C, trap cooling temp: -10 °C, Trap desorb temp: 280 °C, pressurizing gas pressure: 192 kPa, Equilibrium time: 15 min</td></tr>
<tr><td>Oven temperature program</td><td colspan="4">35 °C (5 min hold); ramped at 20 °C/min to 235 °C (5 min hold)</td></tr>
<tr><td colspan="5">Mass Spectrometric parameters</td></tr>
<tr><td>MS parameters</td><td colspan="4">Ionization mode: Electron Ionization<br>Transfer line temperature: 230 °C<br>Ion source temperature: 230 °C</td></tr>
<tr><td>MRM transitions</td><td>Retention time (min)</td><td>Precursor ion (m/z)</td><td>Product ion (m/z)</td><td>CE</td></tr>
<tr><td rowspan="3">EO</td><td rowspan="6">After the void volume</td><td>44</td><td>14</td><td>20</td></tr>
<tr><td>44</td><td>28</td><td>5</td></tr>
<tr><td>44</td><td>29</td><td>5</td></tr>
<tr><td rowspan="3">2-CE</td><td>80</td><td>31</td><td>5</td></tr>
<tr><td>80</td><td>43</td><td>5</td></tr>
<tr><td>80</td><td>44</td><td>5</td></tr>
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<br>In the case of the sesame seed matrix, 100 µL of the extract was directly analyzed by HS-GC-MS/MS. For chilli powder, turmeric powder, and guar gum, an aliquot of 1 mL of the supernatant was cleaned by d-SPE with 50 mg of PSA. After centrifugation at 10,000 rpm for 5 min, an aliquot of 100 µL was taken in a 20 mL HS vial and carefully sealed before the final analysis.
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<tr><td>Results</td><td>In this study, a simple and rapid temperature-controlled extraction method was established for the analysis of EO in a wide variety of dry and high-moisture food matrices. The optimized method provided satisfactory homogeneity, sensitivity, accuracy, and precision for both the target compounds in compliance with the method performance criteria of the SANTE/11312/2021 guideline. The LOQ of both compounds was ≤ 0.01 mg/kg.<br>Furthermore, a satisfactory performance in the intra- and interlaboratory validation studies indicates its ruggedness and reproducibility. Owing to its satisfactory performance, this method is recommended for the determination of EO and its reaction product 2-CE in governmental and commercial food</td></tr>
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| | testing laboratories. The method is expected to facilitate EU–India trade in a variety of fresh and processed commodities due to its high reproducibility. |
| **Calculation** | The detected residues were quantified through matrix-matched calibration, and EO (sum) was calculated using the following formula:<br><br>EO (sum) [mg/kg] = EO + 2 – CE × 0.55 (the conversion factor) |
| **LOQ** | The LOQ was set as the lowest concentration at which the results met the method performance evaluation criteria and were estimated using matrix-matched standards. At LOQ, the S/N of the quantifier SRM was >10:1. The recoveries at LOQ were above 70%. |
| **Storage and Safety Precautions** | Following storage and safety precautions shall be taken while handling it:<br>1. EO is highly volatile, and hence it was important to analyze the samples at a temperature of < 10 °C.<br>2. To prevent skin, eye, and inhalation contact, put on the proper protective eyewear and an apron with long sleeves.<br>3. Both EtO and 2-CE are toxic and carcinogenic, thus require butyl rubber gloves for handling. |
| **Reference** | • Analytical quality control and method validation procedures for pesticide residues analysis in food and feed. SANTE/11312/2021.<br>• EN 15662:2018. Foods of plant origin. Multimethod for the determination of pesticide residues using GC- and LC-based analysis following acetonitrile extraction/partitioning and cleanup by dispersive SPE. Modular QuEChERS-method.<br>• Nerpagar A., ……., **Banerjee Kaushik** (2023). Dynamic headspace GC-MS/MS analysis of ethylene oxide and 2-chloroethanol in dry food commodities: a novel approach *Journal of Environmental Science and Health, Part B* https://doi.org/10.1080/03601234.2023.2264740<br>• Patil R., Langade N., ……, **Banerjee Kaushik** (2023). Development and validation of a residue analysis method for ethylene oxide and 2-chloroethanol in foods by gas chromatography tandem mass spectrometry. *ACS Agricultural Science and Technology* https://doi.org/10.1021/acsagscitech.2c00319 |
| **Approved by** | Scientific Panel on Methods of Sampling and Analysis |