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Structural identification of extractables from rubber closures used for pre-filled semisolid drug applicator by chromatography, mass spectrometry, and organic synthesis

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Abstract

Extractables or leachables from rubber closures used for drug products can potentially be detrimental since some of these rubber extractables are known to be toxic and pyrogenic. In addition, these extractables could interfere with analysis or even affect the stability profile of the active ingredients. For these reasons, it became necessary to identify structures of the possible extractables or leachables from rubber closures so that their potential impact on the drug products that are in direct contact with rubber closures could be evaluated. In this report, five species, 4-(1,1-dimethyl-propyl)-phenol (**1**), sulfur (**2**), 2,6-di-*tert*-butyl-[1,4] benzoquinone (**3**), furan-2-yl-(5-hydroxymethyl-furan-2-yl)-methanol (**4**), and 2-bromo-4-(1,1-dimethyl-propyl)-phenol (**5**), were found to be possible extractables from the rubber closures which were used in a selected pre-filled semisolid drug applicator. A strategy combining HPLC, GC, mass spectrometry, and organic synthesis was utilized.

Keywords: Chromatography; Mass spectrometry; Rubber closures extractables; 2-Bromo-4-(1,1-dimethyl-propyl)-phenol; Furan-2-yl-(5-hydroxymethyl-furan-2-yl)-methanol

1. Introduction

It was known that extractables or leachables from rubber closures into drug products can be detrimental since many of the materials extracted from closures are active chemicals which could be toxic, pyrogenic or even affect the stability of the active ingredients or interfere with the assays [1–6]. Regulatory authorities have increased their scrutiny of drug delivery products or medical devices which come into direct or indirect contact with rubber/plastic materials, inks, and adhesives. Drug manufacturers are expected to investigate the possibility of compounds leaching into drug products or the human body and determine any effects on the quality and/or safety of the products. Drug manufacturers are also expected to show due diligence to characterize and identify major leachables or extractables and evaluate the potential toxicity of those compounds [7–11]. In the event that a known toxic compound is found, it will be

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necessary to monitor its level with a validated analytical method. Since excess quantities of accelerators, activators, and other additives are generally used to obtain complete vulcanization of rubber components along with antioxidants and fillers, various quantities of the unreacted components which remain in the rubber stock or their reaction products formed during the rubber manufacturing process may leach into a drug product [12]. It becomes a challenging task to identify the individual leachables or extractables in the drug product since their levels are usually low if present. In this report, the possible extractables from the rubber closures which have been used for a selected applicator containing semisolid drug products were investigated. The purpose of this work was to extract the rubber closures under exaggerated conditions and identify the major extractables which will be used as reference materials to monitor their possible presence in drug products to assure quality. In this report, five extractables were structurally identified, i.e., 4-(1,1-dimethyl-propyl)-phenol (**1**), sulfur (**2**), 2,6-di-*tert*-butyl-[1,4] benzoquinone (**3**), furan-2-yl-(5-hydroxymethyl-furan-2-yl)-methanol (**4**), and 2-bromo-4-(1,1-dimethyl-propyl)-phenol (**5**) using high performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), organic synthesis, and comparison with authentic compounds.

2. Experimental

Authentic 4-(1,1-dimethyl-propyl)-phenol (**1**), sulfur (**2**), and 2,6-di-*tert*-butyl-1,4-benzoquinone (**3**) were obtained from Aldrich (Aldrich, St. Louis, MO, USA) without further purification. HPLC grade acetonitrile and water were obtained from Mallinckrodt (Mallinckrodt, Paris, KY, USA). Rubber closures for the selected pre-filled semisolid drug applicator were obtained from a commercial source. Detailed information about the rubber closures and the drug products will not be disclosed in this report.

High performance liquid chromatography was performed using an Agilent 1100 HPLC system with diode array UV detection set at 220 nm. An analytical reversed phase HPLC method and a semi-preparative reversed phase HPLC method were employed using acetonitrile and water as mobile phases. For the analytical HPLC method, a Prodigy ODS (3) column was used (Prodigy ODS (3), 3 μm, 15 cm × 0.32 cm, Phenomenex, Torrance, CA, USA). The column temperature was 40 °C. A binary gradient was used. Within the first minute, 70% acetonitrile, 1–15 min, linear gradient to 90% acetonitrile, 15–20 min, 90% acetonitrile, and 20–25 min, linear gradient to 70% acetonitrile. The flow rate was 0.6 ml/min. For the semi-preparative HPLC method, a Prodigy ODS (3) column with a larger internal dimension was used (Prodigy ODS (3), 5 μm, 15 cm × 1 cm). The binary gradient profile was the same as that for the analytical HPLC method except that the flow rate was increased to 5 ml/min.

The gas chromatography and mass spectrometry employed a Thermo Finnigan Trace GC-MS system and an Agilent 5890 GC-MSD. The following GC conditions were used. A capillary column was obtained from Restek (Restek Rtx-5, 30 m, 0.25 mm i.d., 10 μm df, Restek, Bellefonte, PA, USA). Helium was used as the carrier gas at flow rate of 1 ml/min. Injection temperature was 200 °C. Detection temperature was 250 °C. The oven temperature was programmed as follows, 100 °C initially for 1 min; then linearly increased to 250 °C at the rate of 10 °C/min. The sample injection volume was 1–4 μl at splitless mode. The MS analyses were performed under both electron ionization (EI) and chemical ionization (CI) conditions. In the EI-MS experiments the electron energy was set at 70 eV and in the CI-MS experiments methane was used as the reagent gas. All MS data were taken in the positive ion mode.

3. Results

3.1. Isolation of the extractables

An exaggerated condition was employed to extract the possible extractables from the rubber closures. Eighteen grams of rubber closures in 33 ml of acetonitrile were heated with a heating mantle and refluxed for 8 h. The acetonitrile solution, i.e., the rubber closures extract, was then used for the structural identification investigation. The analytical HPLC chromatogram of the rubber closures extract is presented in Fig. 1 which indicates that several compounds were extracted from the rubber stoppers. This report will only focus on the structural identification of extractables **1**, **2**, **3**, **4**, and **5** as assigned

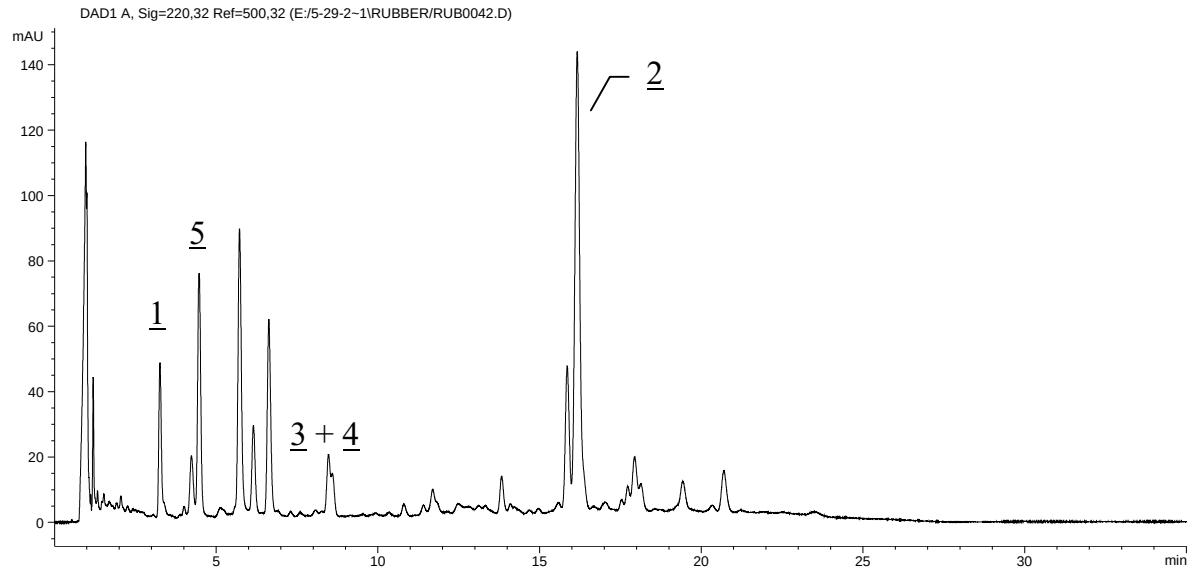


Fig. 1. HPLC chromatograms of the rubber closures extract at 220 nm.

in Fig. 1. Identification of the rest extractables remains to be investigated. Extractables **1**, **2**, **3**, **4**, and **5** were isolated using the semi-preparative HPLC method. Approximately 100–500 μl of the rubber closures extract were injected onto the semi-preparative HPLC column. The fractions containing **1**, **2** and **5** were collected separately. Compounds **3** and **4** were collected as one fraction. The collected fractions containing **1**, **2**, **3**, **4**, and **5** were used for the structural identification investigation without further purification. The original attempts to use HPLC-MS with electrospray (ESI) and atmospheric pressure chemical ionization (APCI) to identify the extractables were not successful due to the unsatisfactory ionization efficiency. An alternative approach using gas chromatography coupled with mass spectrometry was applied and the results are summarized in the following sections.

*3.2. Structural identification of extractable **1***

Extractable **1** was identified to be 4-(1,1-dimethylpropyl)-phenol by interpreting its mass spectrum and by performing a search to known Mass Spectra Libraries (NIST98 Mass Spectral Library). The collected fraction containing **1** was introduced into the mass spectrometer via a GC inlet. The EI mass spectrum of **1** exhibited ions at *m*/*z* 164 (*M*), 149 ($M - CH_3$), 135 ($M - C_2H_5$), 119 ($M - C_2H_5 - CH_4$), and 107 ($M - C_2H_5 - C_2H_4$). In addition, the structure of **1** was also confirmed by its practically identical EI mass spectrum, UV spectrum, and HPLC retention time compared with that of an authentic compound under identical experimental conditions. Compound **1** eluted at 3.3 min by the analytical HPLC method. The UV spectrum of **1** in the HPLC mobile phase exhibited absorption peaks at 223 and 278 nm with an absorption ratio of 1:0.23.

*3.3. Structure identification of extractable **2***

Extractable **2** was identified to be sulfur by comparing its HPLC retention time and UV spectrum with that of an authentic compound under the identical experimental conditions. Compound **2** eluted at 16.2 min by the analytical HPLC method. The UV spectrum of **2** in the HPLC mobile phase exhibited an absorption peak at 225 nm and a broad peak at 265–280 nm with an absorption ratio of 1:0.59.

*3.4. Structural identification of extractable **3** and **4***

Extractable **3** and **4** were initially isolated as a mixture using the semi-preparative HPLC method. To

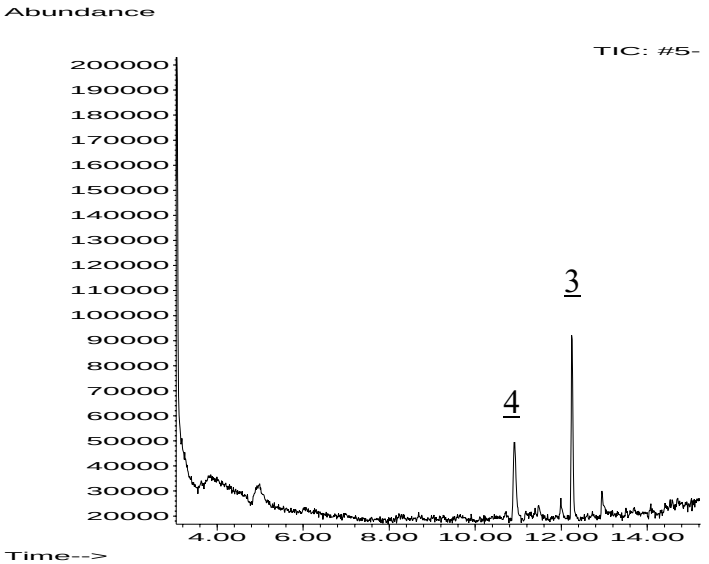


Fig. 2. Full scan total ion chromatogram of the collected fraction containing **3** and **4** from semi-preparative HPLC isolation of the rubber closures extract.

separate them from each other, the collected fraction containing **3** and **4** was introduced into the GC-MS systems with EI and CI capabilities. The EI total ion chromatogram is presented in Fig. 2 which indicates that **3** and **4** were well separated. Extractable **3** was identified to be 2,6-di-*tert*-butyl-[1,4] benzoquinone by interpreting its mass spectrum and by performing search to known Mass Spectra Libraries. The EI mass spectrum of **3** exhibited ions at *m*/*z* 220 (*M*), 205 ($M - CH_3$), 192 ($M - CO$), 177 ($M - CH_3 - CO$), 163 ($M - (CH_3)_3C$), 149 ($M - CH_3 - 2CO$), 135 ($M - (CH_3)_3C - CO$), 107 ($M - (CH_3)_3C - 2CO$), 121 ($M - CH_3 - CO - (CH_3)_2CCH_2$), 91 ($M - CH_3 - 2CO - (CH_3)_3CH$), and 77 ($M - (CH_3)_3C - CO - (CH_3)_3CH$). The structure of **3** was also confirmed by its practically identical EI mass spectrum and HPLC retention time compared with that of an authentic compound under identical experimental conditions. Compound **3** eluted at 8.5 min by the analytical HPLC method.

Extractable **4** was tentatively proposed to be furan-2-yl-(5-hydroxymethyl-furan-2-yl)-methanol based on interpretation of the mass spectrum. The CI mass spectrum of **4** is presented in Fig. 3 which reveals that **4** has a protonated molecular ion at *m*/*z* 195 with a fragment ion at *m*/*z* 177, indicating the propensity for **4** to lose a water molecule. Additional fragmentation ions at *m*/*z* 137, 123, and 109 were observed as well, which were possibly due to the neutral loss of 40, 54 and 68 units from the ion at *m*/*z* 177. The EI mass spectrometric studies show that **4** exhibits a predominant peak at *m*/*z* 97 and lacks the molecular ion peak at *m*/*z* 194 (Fig. 4), indicating a facile mass-symmetric fragmentation occurred in the EI ionization source. The proposed mass spectrometric fragmentation mechanism of **4** is presented in Scheme 1.

3.5. Structural identification of extractable 5

Extractable **5** was determined to be 2-bromo-4-(1,1-dimethyl-propyl)-phenol. The collected fraction containing **5** from semi-preparative HPLC was introduced into the mass spectrometers via a GC inlet. The CI and EI mass spectra of **5** are presented in Figs. 5 and 6, respectively. The CI mass spectrum of **5** exhibits a dominant peak cluster at *m*/*z* 243, which is responsible for the protonated molecular ion containing one bromine atom. The EI-MS spectrum of **5** shows a positive molecular ion at *m*/*z* 242 with a typical ion cluster containing one pair of bromine isotopes with masses of 79 and 81 to give the isotope peaks at *m*/*z* 242 and 244 at nearly equal abundance. The proposed

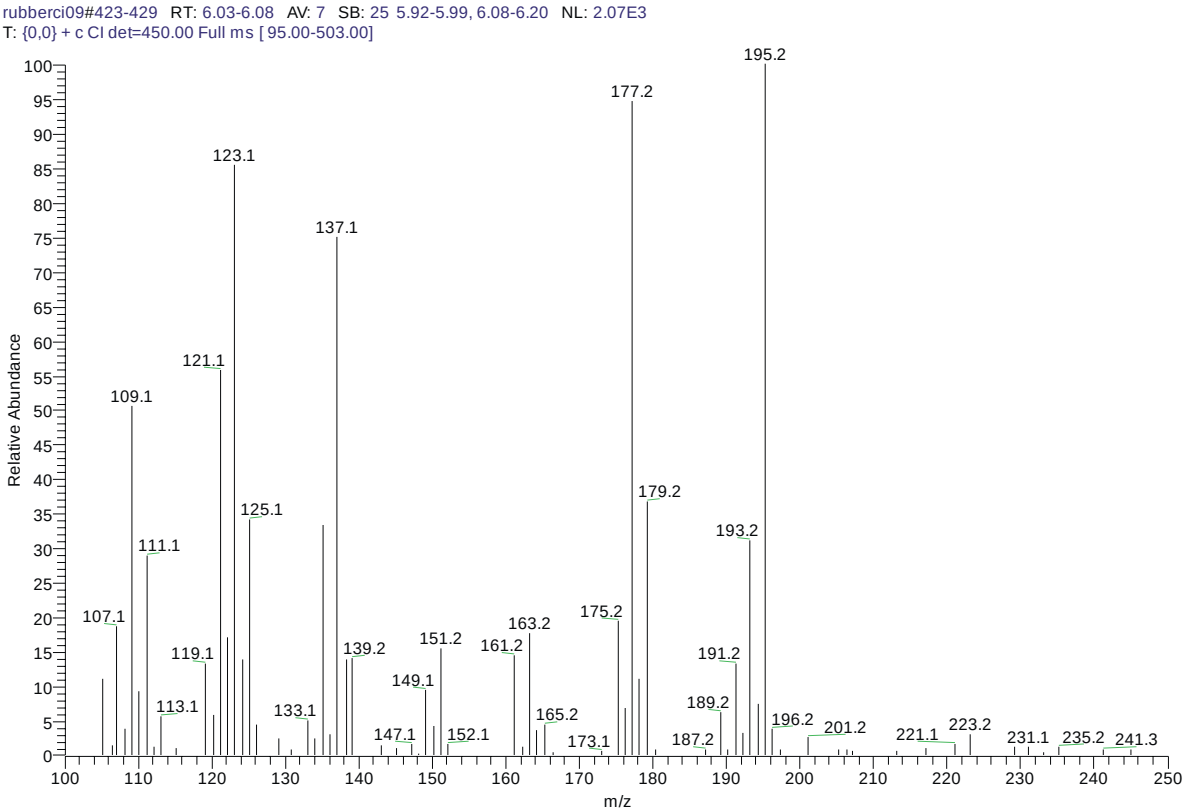


Fig. 3. Mass Spectrum of **4** in chemical ionization mode.

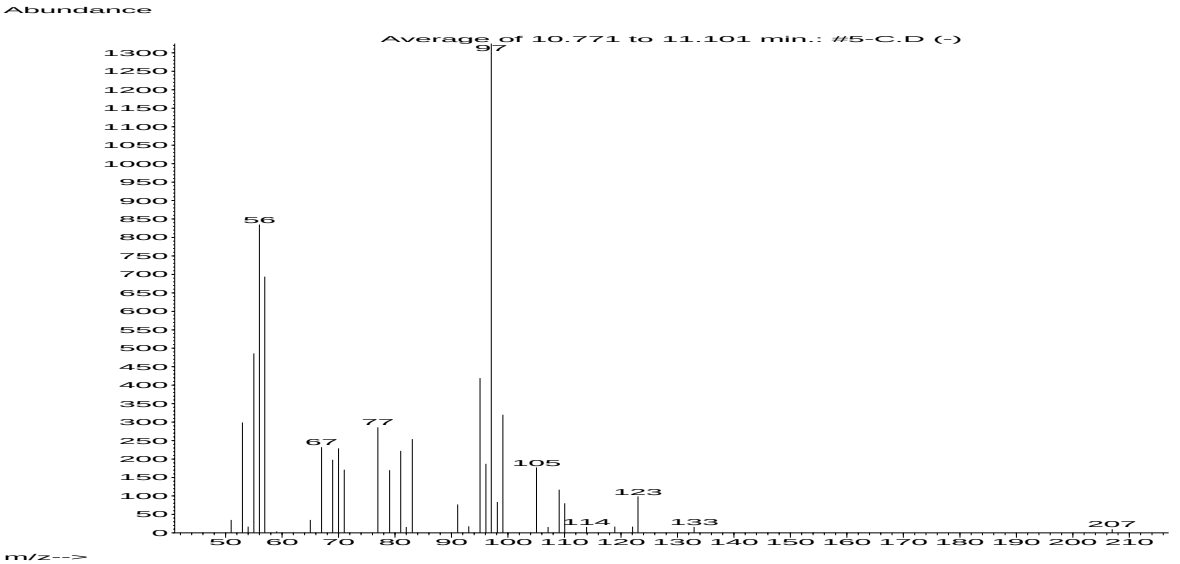
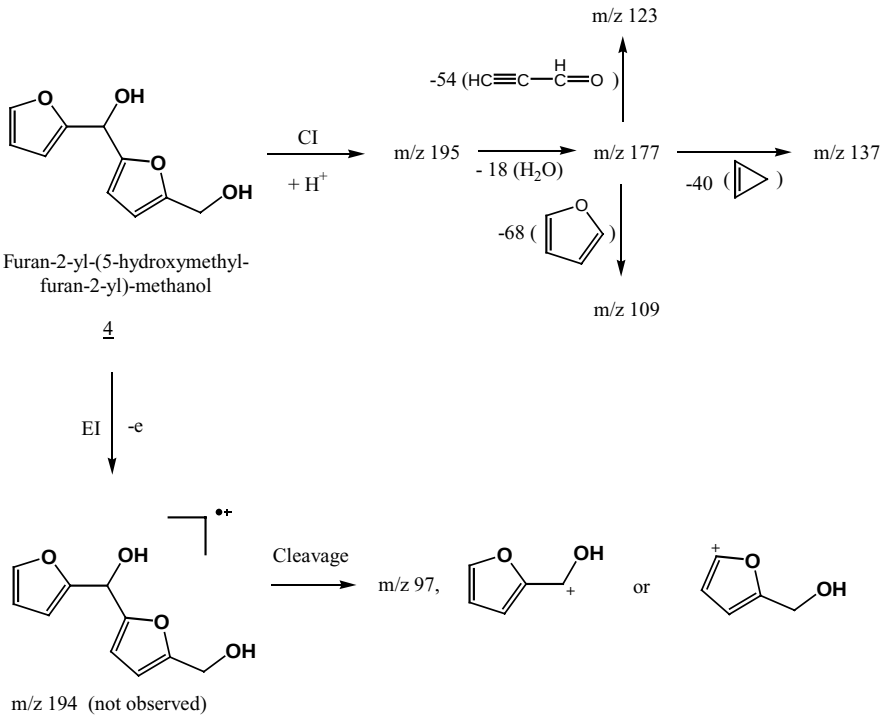


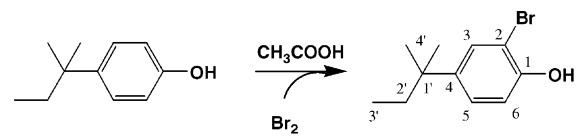
Fig. 4. Mass spectrum of **4** in electron impact ionization mode (EI).



Scheme 1. Proposed mass spectrometric fragmentation pathway of **4**.

mass spectrometric fragmentation pattern is presented in Scheme 2.

To confirm the structure of extractable **5**, authentic 2-bromo-4-(1,1-dimethyl-propyl)-phenol was synthesized chemically since it is not commercially available. 2-Bromo-4-(1,1-dimethyl-propyl)-phenol was synthesized by bromination of 4-(1,1-dimethyl-propyl)-phenol in acetic acid and then purified by the semi-preparative HPLC method. The synthetic method was modified based on the report by Romadane and Chizhikova [13]. The structure of the synthesized 2-bromo-4-(1,1-dimethyl-propyl)-phenol was confirmed by EI-MS, and NMR including ^{1}H, ^{13}C and 2D analyses. Its purity was established using quantitative NMR evaluation.



4-(1,1-dimethyl-propyl)-phenol

1

2-Bromo-4-(1,1-dimethyl-propyl)-phenol

5

Four grams of 4-(1,1-dimethyl-propyl)-phenol was dissolved in 40 ml acetic acid followed by dropwise addition of 1.4 ml bromine (∼5 min needed) at room temperature. The reaction solution was kept at room temperature for 30 min and then was purged with nitrogen to remove the solvent. After addition of 6 ml methanol to reconstitute the residual, aliquots of the solution were repeatedly injected into the semi-preparative HPLC column for isolation. The synthesized compound which eluted at ∼6 min was collected. The solvent in the collected fraction was removed using a rotary evaporator at 45 °C under vacuum to give a light yellow viscous liquid. After drying over P_2O_5 under vacuum for more than 24 h, ∼2.5 g material was obtained, which was then subjected to MS and NMR analysis. The EI-MS spectrum gave a positive molecular ion at *m/z* 242 with a typical ion cluster containing one pair of bromine isotopes with masses of 79 and 81 to give the isotope peaks at *m/z* 242 and 244 at nearly equal abundance and other expected ions. ^{1}H, ^{13}C, HMBC (^{1}H–^{13}C heteronuclear multiple bond coherence), and HMQC

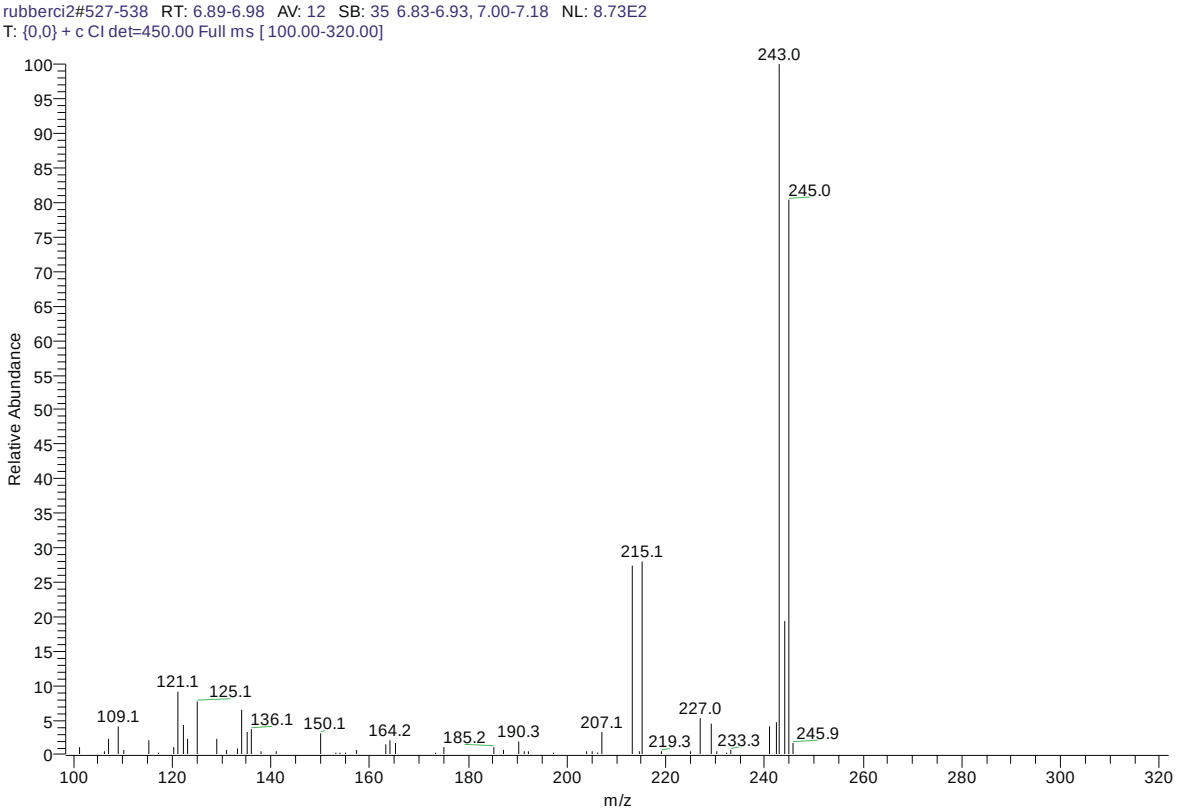


Fig. 5. Mass spectrum of **5** in chemical ionization mode (CI).

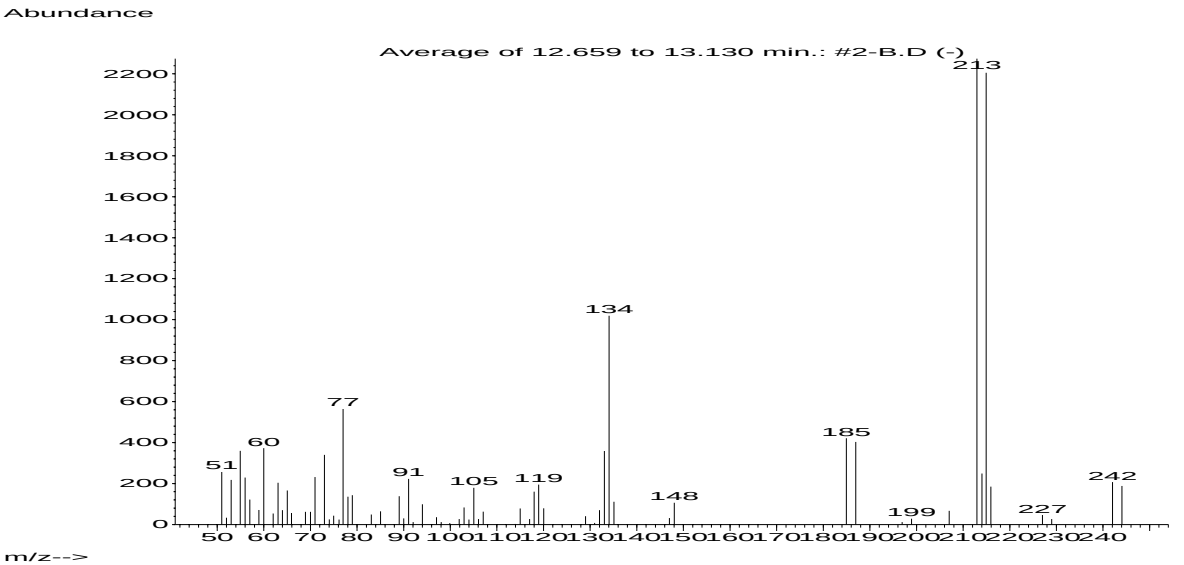
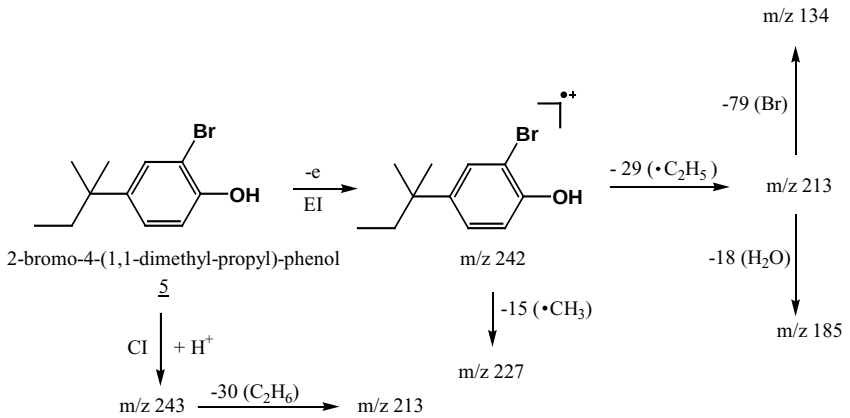


Fig. 6. Mass spectrum of **5** in electron impact ionization mode (EI).



Scheme 2. Proposed mass spectrometric fragmentation pathway of **5**.

(^{1}H–^{13}C heteronuclear multiple quantum coherence) NMR measurements were performed. The following ^{1}H NMR (DMSO-d_6) signals were observed—σ, 9.938 (s, 1H, OH), 7.334 (d, $J = 2.4$ Hz, 1H, C(3)-H), 7.125 (dd, $J = 8.4$ Hz, $J = 2.4$ Hz, 1H, C(5)-H), 6.882 (d, $J = 8.4$ Hz, 1H, C(6)-H), 1.542 (q, $J = 7.4$ Hz, 2H, C(2′)-H_2), 1.180 (s, 6H, 2C(4′)-H_3), and 0.608 (t, $J = 7.4$ Hz, 3H, C(3′)-H_3). The following ^{13}C NMR (DMSO-d_6) signals were observed—σ, 151.560 (C(1)), 141.154 (C(4)), 129.877 (C(3)), 126.077 (C(5)), 115.900 (C(6)), 108.881 (C(2)), 36.882 (C(1′)), 36.122 (C(2′)), 28.276 (C(4′)), and 8.992 (C(3′)). ^{1}H–^{13}C HMQC indicated that the following pairs of ^{1}H and ^{13}C resonances correlated to each other. 7.334 (C(3)-H) and 129.887 (C(3)), 7.125 (C(5)-H) and 126.077 (C(5)), 6.882 (C(6)-H) and 115.900 (C(6)), 1.542 (C(2′)-H_2) and 36.122 (C(2′)), 1.180 (2C(4′)-H_3) and 28.276 (2C(4′)), as well as 0.608 (C(3′)-H_2) and 8.992 (C(3′)). ^{1}H–^{13}C HMBC indicated that, in addition to the expected 2-bond correlations, the following 3-bond correlations for the pairs of ^{1}H and ^{13}C resonances were observed—7.334 (C(3)-H) and 151.560 (C(1)), 7.334 (C(3)-H) and 126.077 (C(5)), 7.334 (C(3)-H) and 36.882 (C(1′)), 7.125 (C(5)-H) and 151.560 (C(1)), 7.125 (C(5)-H) and 129.877 (C(3)), 7.125 (C(5)-H) and 36.882 (C(1′)), 6.882 (C(6)-H) and 141.154 (C(4)), 6.882 (C(6)-H) and 108.881 (C(2)), 1.542 (C(2′)-H_2) and 141.154 (C(4)), 1.542 (C(2′)-H_2) and 28.276 (C(4′)), 1.180 (2C(4′)-H_3) and 141.154 (C(4)), 1.180 (2C(4′)-H_3) and 36.122 (C(2′)), 1.180 (C(4′)-H_3) and 28.276 (C(4′)), 0.608 (C(3′)-H_3) and 36.882 C(1′)). The purity of the synthesized compound was estimated to be 98.9% by quantitative ^{1}H NMR analysis. This measurement was performed using maleic acid as internal standard and DMSO-d_6 as solvent. The quantitation was achieved by comparing the resonance for protons at C(4′) in the synthesized 2-bromo-4-(1,1-dimethyl-propyl)-phenol and the resonance of the alkene protons in maleic acid. The analysis was performed with duplicate sample weights.

Extractable **5** from the rubber closures extract was confirmed to be 2-bromo-4-(1,1-dimethyl-propyl)-phenol by its practically identical mass spectrum, UV spectrum, and HPLC retention time compared with that of the synthesized authentic compound under the same conditions. Compound **5** eluted at 4.5 min when analyzed by the analytical HPLC method; its UV spectrum in the HPLC mobile phase exhibited absorption at 220 (sh) and 284 nm with an absorption ratio of 1:0.21.

4. Discussion

The compounds 4-(1,1-dimethyl-propyl)-phenol, sulfur, 2,6-di-*tert*-butyl-[1,4] benzoquinone, furan-2-yl-(5-hydroxymethyl-furan-2-yl)-methanol, and 2-bromo-4-(1,1-dimethyl-propyl)-phenol were found to be possible extractables from the selected rubber closures of the semisolid drug applicator. An analytical strategy with a combination of analytical and

semi-preparative HPLC, GC-MS and organic synthesis proved to be effective in solving this complicated problem. A new synthetic and purification procedure for 2-bromo-4-(1,1-dimethyl-propyl)-phenol was established and its purity was also established using quantitative NMR measurements. Monitoring the levels of these identified species, if present, in the drug products and evaluating their toxicity could provide additional assurance for the quality of the drug products.

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