Mass Spectral Fragmentation of the Intravenous Anesthetic Propofol and Structurally Related Phenols

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Propofol (2,6-diisopropyl phenol) is a widely used intravenous anesthetic. To define its pharmacokinetics and pharmacodynamics, methods for its quantitation in biological matrixes have been developed, but its pattern of mass spectral fragmentation is unknown. We found that fragmentation of the $[M - H]^-$ ion (*m/z* 177) of propofol in both APCI MS/MS and ESI MS/MS involves the stepwise loss of a methyl radical and a hydrogen radical from one isopropyl side chain to give the most intense product ion, $[M - H - CH_4]^-$, at *m/z* 161. This two-step process is also the preferred mode of fragmentation for similar branched alkyl substituted phenols. This mode of fragmentation of the $[M - H]^-$ ion is supported by three independent lines of evidence: (1) the presence of the intermediary $[M - H - CH_3]^-$ radical ion under conditions of reduced collision energy, (2) the determination of the mass of the predominant $[M - H - CH_4]^-$ product ion by high resolution mass spectrometry, and (3) the pattern of product ions resulting from further fragmentation of the $[M - H - CH_4]^-$ product ion. Phenols with a single straight chain alkyl substituent, in contrast, undergo β elimination of the alkyl radical irrespective of the length of the alkyl chain, yielding the most intense product ion at *m/z* 106. This product ion represents a special case of a stable intermediary radical for the two-step process described for branched side chains, because further elimination of a hydrogen radical from the β carbon is not possible. (J Am Soc Mass Spectrom 2005, 16, 814–824) © 2005 American Society for Mass Spectrometry

Propofol (2,6-diisopropyl phenol) is an intravenous anesthetic with a phenolic structure. It is used for both induction [1] and maintenance [2] of general anesthesia. It is also useful for sedation [3] as a supplement to regional anesthesia and in critically ill patients confined to intensive care units. The patient loses consciousness 30 to 50 s after receiving the drug and remains asleep for about 4 to 6 min [1, 2]. Even though a single dose of propofol has a short duration of action and produces fast recovery from anesthesia, propofol is not metabolized in a few minutes. It is present in blood, adipose tissues, liver, and brain for a few hours, its concentration decreasing with time [4]. To study this tissue distribution

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and the pharmacokinetics of propofol, it is necessary to quantitate it precisely.

Various methods have been reported for the quantitation of propofol in plasma or blood: high performance liquid chromatography (HPLC) with ultraviolet absorbance [5], fluorescence [6], or electrochemical detection [7]; gas chromatography (GC) with atomic emission [8] or mass spectrometric (MS) detection [9]; capillary GC or head-space GC [10] with solid-phase microextraction. The coupling of a MS detector to the HPLC system offers a more selective analytical technique compared with HPLC methods using traditional detectors. We attempted to develop an LC/MS method for the detection of propofol, but observed interfering peaks at the propofol retention time and a high background while applying the method to human plasma samples. A second stage of mass analysis (MS/MS) enhanced selectivity and provided an improved signal-to-noise ratio compared with single-stage MS. This technique also reduced the need for complete resolution of the analyte from the endogenous matrix compared to HPLC with other modes of detection. We recently reported this LC/MS/MS method for detection and quantitation of propofol in human plasma [11].

Phenols are known to readily form negative ions in the gas phase, and there are several reports on their negative ion chemical ionization mass spectra [12–14]. One report on the fragmentation of phenolic anions in the gas phase is restricted to phenol only [12]. Reports on negative ion LC/MS for the identification of propofol using APCI for ionization fail to discuss its fragmentation [15, 16]. Fragmentation of the $[M + H]^+$ ion of sterically hindered phenols formed by chemical ionization using methane or butane as the reagent gas [17, 18] indicate the formation of product ions by the loss of a methyl radical at the β carbon of substituent side chains. There are some reports on the gas-phase decomposition of alkoxide ions generating hydrogen and methane as the main fragmentation by-products [19–21]. Based on a deuterium isotope effect study, Brauman and coworkers reported that the infrared multiphoton-induced elimination of methane or hydrogen from the *t*-butoxide negative ion proceeds by a stepwise mechanism [19, 20] involving bond cleavage as the first step and a subsequent hydrogen transfer reaction from an intermediate ion-molecule complex. This mechanism was further supported in a study of isotope effects using a sector mass spectrometer and ab initio calculations [21]. During development of our LC/MS/MS method for the determination of propofol that used negative ion atmospheric pressure chemical ionization (APCI), we observed a fragmentation pattern that was entirely different from that reported for other phenols [22]. Therefore, we undertook a detailed study of the mass spectral fragmentation of the $[M - H]^-$ ions of propofol and structurally related phenols in APCI and electrospray ionization (ESI) modes and confirmed the mass of the proposed $[M - H - CH_4]^-$ product ion using high resolution mass spectrometry.

Experimental

Chemicals

Propofol and most phenols used in this study were purchased from Sigma-Aldrich Co. (St. Louis, MO). 4-Vinyl phenol, 4-n butyl phenol, 4-n pentyl phenol, and 4-n heptyl phenol were purchased from Lancaster, Inc. (Windham, NH). HPLC-grade methanol was purchased from Fischer Scientific Co. (Hampton, NH).

Mass Spectrometry

All spectra were recorded in negative ion mode, as phenols are much less sensitive towards ionization in positive ion mode by the addition of a proton. APCI and ESI spectra were acquired on three different mass spectrometers, a triple quadrupole instrument, a hybrid quadrupole/time of flight instrument, and a quadrupole ion trap instrument as described below.

Triple Quadrupole Tandem Mass Spectrometry

Most of the spectra were collected with a triple quadrupole mass spectrometer (API 4000, Applied Biosystems/MDS Sciex, Foster City, CA) equipped with the manufacturer's APCI or ESI probe (also described as turbo ion spray by the manufacturer). APCI conditions were a vaporizer temperature of 250 °C and a corona discharge current of −5 μA. Nitrogen supply pressures were 30, 48, and 6 psi for curtain, nebulizing, and collision gas, respectively. Collision energies ranged from 30 to 45 eV and declustering potentials from −68 to −88 V. ESI conditions were a vaporizer temperature of 300 °C, an ion spray voltage of −4500 V, and a declustering potential of −68 to −88 V. Nitrogen supply pressures were 45, 48, and 6 psi for nebulizing, auxiliary, and collision gas, respectively. Collision energies ranged from 30 to 45 eV.

Spectra were recorded using scan ranges suitable for each particular analyte. Product ion scans were recorded from *m/z* 50 to a value approximately 10% greater than that of the parent ion. Normal MS scans covered a range from *m/z* 50 to a value twice the molecular weight of the analyte. Parent ion scans were acquired over a limited scan range of *m/z* 100 to 200, with masses set appropriately for the ions of interest. Scan rates for both normal mass and product ion spectra were typically on the order of 200 *m/z* units per second. Samples were prepared in methanol at an approximate concentration of 500 ng/ml. They were introduced into the ion source at a flow rate of 40 μL/min by using a syringe pump (Harvard model 22, Harvard Apparatus, Holliston, MA). Spectra were acquired in profile mode with a 1 s acquisition

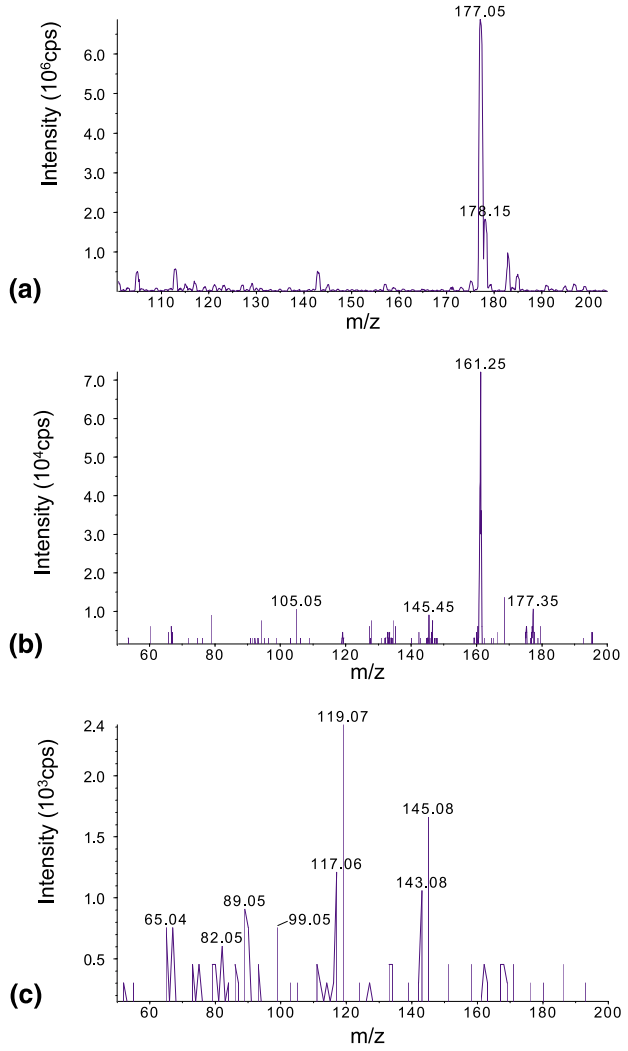


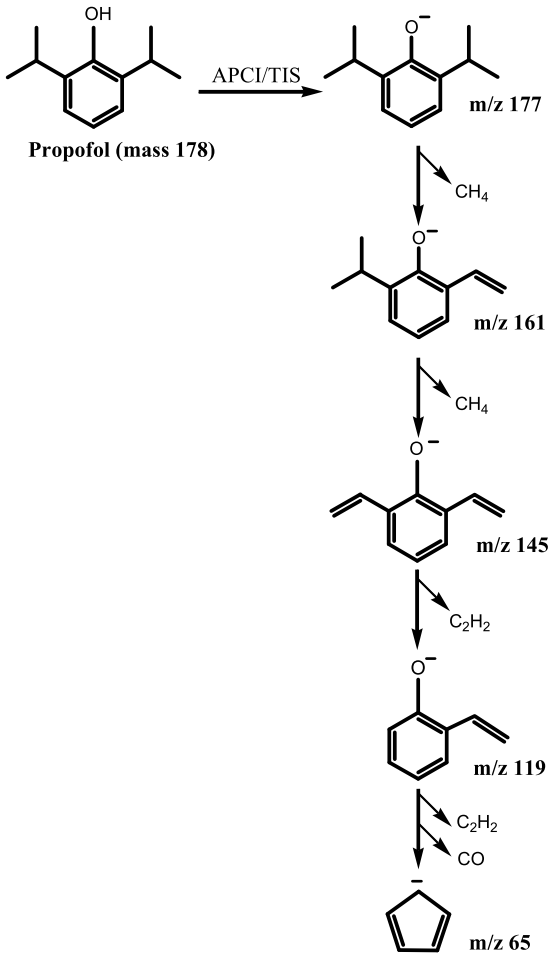
Figure 1. Mass spectral fragmentation of the $[M - H]^-$ ion of propofol with APCI in negative ion mode on a triple quadrupole mass spectrometer. (**a**) Normal mass spectrum; (**b**) product ion spectrum of *m/z* 177, dominated by a single product at *m/z* 161; (**c**) product ion spectrum of *m/z* 161; *m/z* 161 was selected with quadrupole 1 and further fragmented.

duration typically by summation of 10 individual scans.

HPLC/Quadrupole Ion Trap Tandem Mass Spectrometry

The second mass spectrometer was a ThermoFinnigan (San Jose, CA) LCQ-quadrupole ion trap mass spectrometer. Tandem mass spectrometry data were obtained with both negative ESI and negative APCI. For ESI instrument settings were: spray voltage 3.3 kV, nitrogen sheath gas 60 (instrument parameter), auxiliary gas 5, heated capillary voltage −15 V, temperature 250 °C, and tube lens offset 20 V. For APCI, the vaporizer temperature was 300 °C, the heated capillary temperature was 250 °C, the discharge needle was operated at 6.0 kV and 6.0 μA. The other settings were nitrogen sheath gas 25, auxiliary gas 0, heated capillary voltage 15 V, and tube lens offset 0 V. Tandem mass spectra were obtained utilizing the advanced features of the scan function. In general, the ions were isolated with a 2.5 or 3.0 u window and then collision-induced dissociation (CID) was performed at a Mathieu q of 0.4 or 0.45 with a resonant excitation voltage of 43 to 47% maximum (software parameter) applied for 30 ms. For some weak MS_n spectra, the isolation window widths for the earlier generation product ions were increased to increase the sensitivity.

Sample introduction was via HPLC utilizing an Agilent (Palo Alto, CA) 1100 series binary pump with mobile phase A being H_2O and mobile phase B being methanol. The HPLC flow rate was 0.15 mL/min. A reverse-phase gradient liquid chromatographic separation [A:B(min) = 95:5(0) to 5:95(45–60)] was initially performed on a Phenomenex (Torrace, CA) Synergi 4μ Hyrdro-RP 80 A column (2 mm × 150 mm) with a C_{18} guard column (2 mm × 4 mm) to ascertain purity. As no significant mass spectrometric interferences were found, the remaining analyses were performed with the C_{18} guard column men-



Scheme 1

Table 1. Mass spectral fragmentation of propofol and structurally related phenols

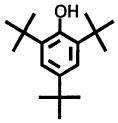
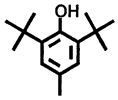
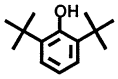
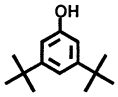
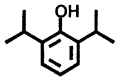
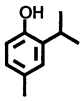
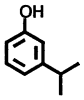
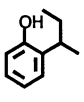
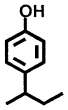
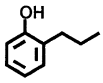
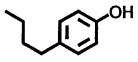
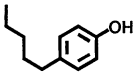
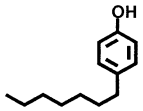
Sample number	Structure	Phenol	Molecular weight (g/mol)	Quasi-molecular Ion $[M-H]^-$ (*m/z*)	Most abundant product ion (*m/z*)	Elimination product (mass)
1		2,4,6-Tritertiary butyl phenol	262.4	261	245.4	15.6
2		2,6-Ditertiary butyl-4-methyl phenol	220.4	219.1	203.1	16
3		2,6-Ditertiary butyl phenol	206.3	205.1	189.2	15.9
4		3,5-Ditertiary butyl phenol	206.3	205.1	189.2	15.9
5		2,6-Diisopropyl phenol (propofol)	178.3	177.1	161.2	15.9
6		2-Isopropyl-4-methyl phenol	150.2	149.2	133.1	16.1
7		2-Isopropyl phenol	136.2	135	119.1	15.9
8		3-Isopropyl phenol	136.2	135	119.1	15.9
9		2-Tertiary butyl phenol	150.2	149.1	133.1	16
10		3-Tertiary butyl phenol	150.2	149.2	133.1	16.1
11		4-Tertiary butyl phenol	150.2	149.1	133.1	16
12		4-Tertiary amyl phenol	164.3	162.9	133.1	29.8
13		2-Secondary butyl phenol	150.2	149.2	119.1	30.1

Table 1. Mass spectral fragmentation of propofol and structurally related phenols

Sample number	Structure	Phenol	Molecular weight (g/mol)	Quasi-molecular Ion $[M\text{-}H]^-$ (*m/z*)	Most abundant product ion (*m/z*)	Elimination product (mass)
14		4-Secondary butyl phenol	150.2	149.1	119.1	30
15		2-Ethyl phenol	122.2	120.9	106.2	14.7
16		2-n-Propyl phenol	136.2	135	106	29
17		4-n-Butyl phenol	150.2	149.1	105.8	43.3
18		4-n-Pentyl phenol	164.3	162.9	106.2	56.7
19		4-n-Heptyl phenol	192.3	190.9	106.3	84.6
20		2-Hydroxy phenol (catechol)	110.1	107.8	80.3	27.5
21		2-Methyl phenol	108.1	106.9	92.3	14.6
22		3-Methyl phenol	108.1	106.8	92.3	14.5
23		4-Methyl phenol	108.1	106.8	92.3	14.5
24		4-Vinyl phenol	120.2	119.1	93.1	26

tioned above and an isocratic separation (0.15 mL/min of A:B = 15:85).

Hybrid Quadrupole/Time of Flight High Resolution Mass Spectrometry

Precise determination of the mass of product ions was carried out on a third mass spectrometer, an API QSTAR Pulsar hybrid quadrupole time-of-flight (QTOF) mass spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA) equipped with a Protana nanospray source (Protana Engineering A/S, Starmosegardsvej, Denmark). Normal mass spectra and product ion mass spectra were acquired in negative ion electrospray mode and externally calibrated against $[M - H]^-$ and SO_3^- fragment ions of a taurocholic acid standard at *m/z* 514.2838 and 79.9568, respectively. Calibration allowed for mass accuracies of <30 ppm. For collision-induced dissociation (CID), precursor ion selection was performed using the

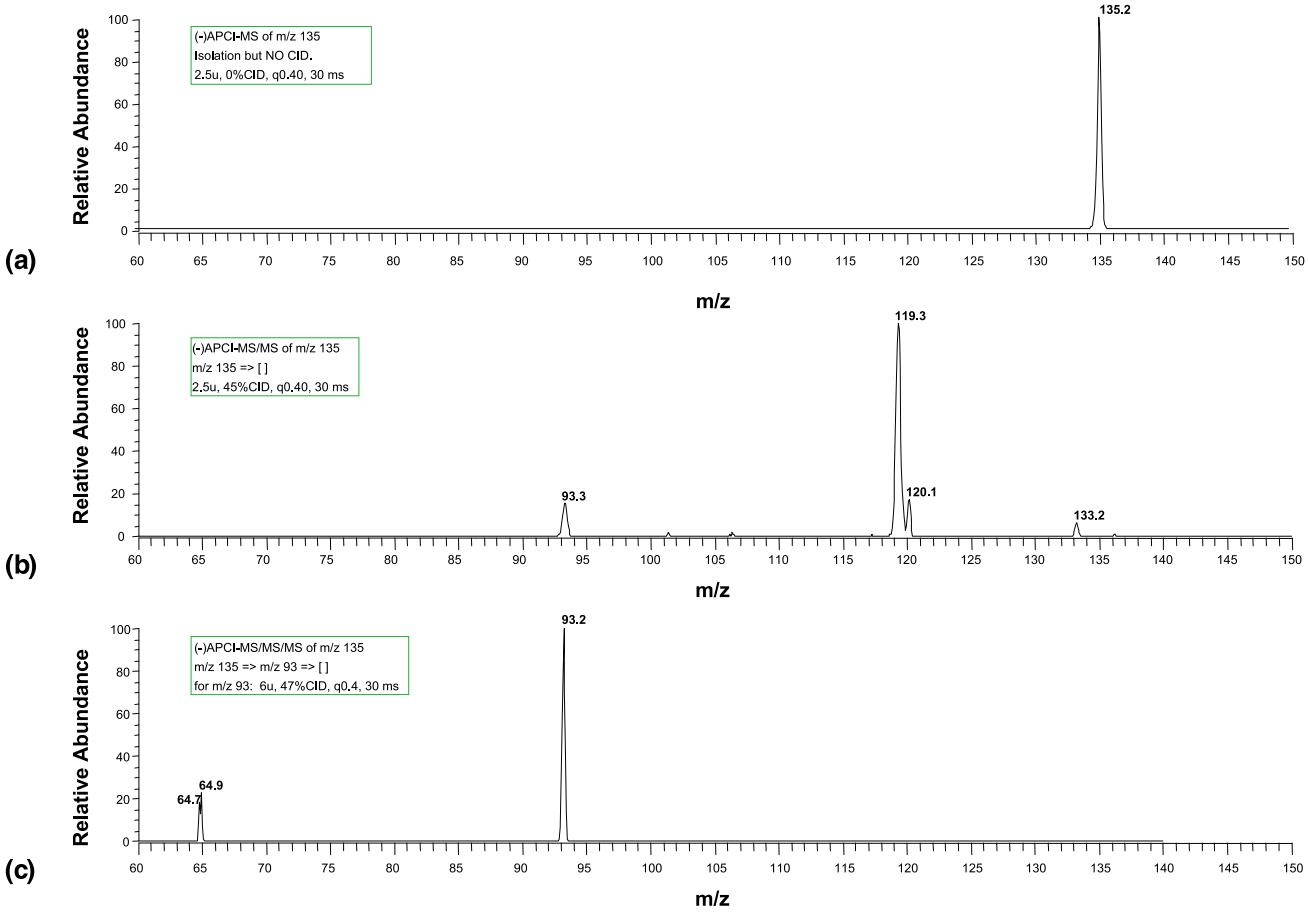


Figure 2. Mass spectral fragmentation of the $[M - H]^-$ ion of 2-isopropyl-phenol with APCI in negative ion mode. (**a**) Normal mass spectrum (0% CID); (**b**) product ion spectrum at 45% CID yields product ions at *m/z* 93, 119, 120, and 133; (**c**) further fragmentation of *m/z* 93 yields *m/z* 65. These spectra were recorded with a Finnigan LCQ mass spectrometer.

quadrupole mass filter. Selected ions were fragmented in a subsequent quadrupole collision cell using nitrogen as the collision gas. Collision energy was 35 eV.

Results and Discussion

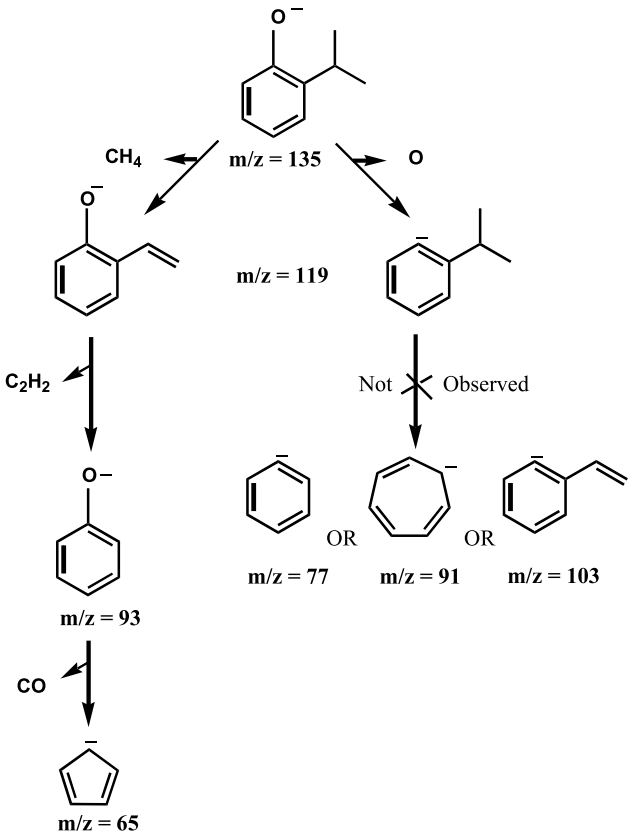
Limited information is available on the fragmentation of simple substituted phenols such as propofol. While published mass spectrometric methods for quantitation of propofol describe the parent-product ion pair used to identify and quantify propofol, they neither provide details of the fragmentation pattern nor address the mechanism of the fragmentation [8, 9, 15, 16]. Because the fragmentation behavior of the $[M - H]^-$ ion of propofol using APCI and ESI probes was not consistent with the expected loss of a methyl radical at the β carbon of the isopropyl side chain, we performed a detailed study of the mass spectral fragmentation of propofol and structurally related phenols, both in the positive as well as negative ion mode. Our investigation revealed a unique and interesting fragmentation of these simply structured ions.

Positive Ion Study

Propofol was much less sensitively ionized in the positive ion mode than in the negative ion mode by either APCI or ESI [15]. Similarly none of the other phenols used in this study was ionized efficiently in the positive ion mode. At typical working concentrations of 500 ng/ml used in this study the positive ion mode did not produce a detectable ion signal. These results are consistent with studies on the positive ion chemical ionization of phenols that showed that ionization by proton addition was inefficient due to the sterically crowded hydroxyl group as well as the slightly acidic nature of these phenols [15, 16].

Negative Ion Study

In the negative ion mode, the product ion spectrum for propofol's $[M - H]^-$ ion (*m/z* 177) revealed an interesting fragmentation behavior (Figure 1). Although the $[M - H]^-$ ions of substituted phenols are typically easily fragmented and yield information-rich mass spectra, the product ion spectrum of *m/z* 177 was



Scheme 2

noteworthy for the paucity of fragmentation products. It displayed only a single major product ion at *m/z* 161. All other product ions were nearly tenfold less abundant. Such a predominance of the ion *m/z* 161 was found despite the use of APCI MS/MS conditions that allow major decomposition processes, namely collision energies from 25 to 30 eV and nitrogen pressures sufficient for multiple collisions.

Even more interesting was the characterization of the neutral loss. In the case of alkyl substituted phenols, cleavage of the β carbon bond of the substituent to the phenyl ring is well documented [22] and always gives the major fragment ion. Thus, the loss of a methyl radical from the isopropyl side chain was expected to dominate the product ion spectrum of propofol. The major elimination product (nominal mass 16), however, is not consistent with the loss of a methyl radical (nominal mass 15). The loss of 16 mass units could be explained by the loss of either a methane molecule or an oxygen atom from the $[M - H]^-$ parent ion. To ascertain the structure of the product ion (*m/z* 161), the abundance of *m/z* 161 was increased by raising the declustering potential to −90 V to induce so-called in-source fragmentation. Subsequent fragmentation at a collision energy of 45 eV resulted in a mass spectrum with major identifiable peaks at *m/z* 145, 119, and 65 (Figure 1c) which correspond to the fragmentation products of the $[M - H]^-$ ion of propofol shown in Scheme **1** and are corroborated by the fragmentation experiments on 2-isopropyl phenol discussed below. Because of less abundance of ions at *m/z* 143, 89, and 67, these were not assigned a structure.

To ascertain the identity of the proposed neutral loss (*m/z* 16) in the fragmentation of propofol's $[M - H]^-$ ion, structurally related phenols were subjected to CID under conditions similar to those for propofol. Some highly substituted phenols required higher collision energy from 45 to 55 eV for fragmentation. The results of these CID studies are shown in Table 1. Phenols that have only methyl groups in the β position of the substituent (sample numbers 1–11) all show the most prominent product ion consistent with a neutral loss of

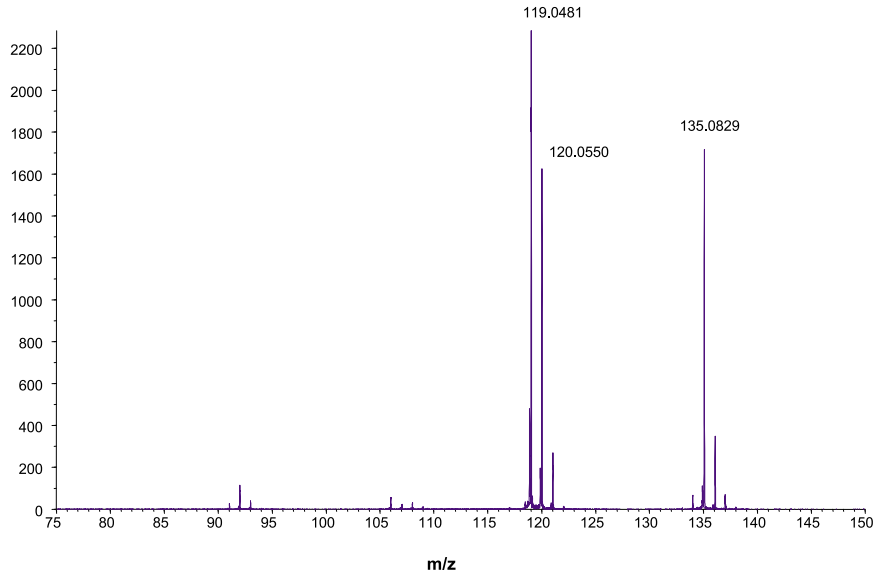
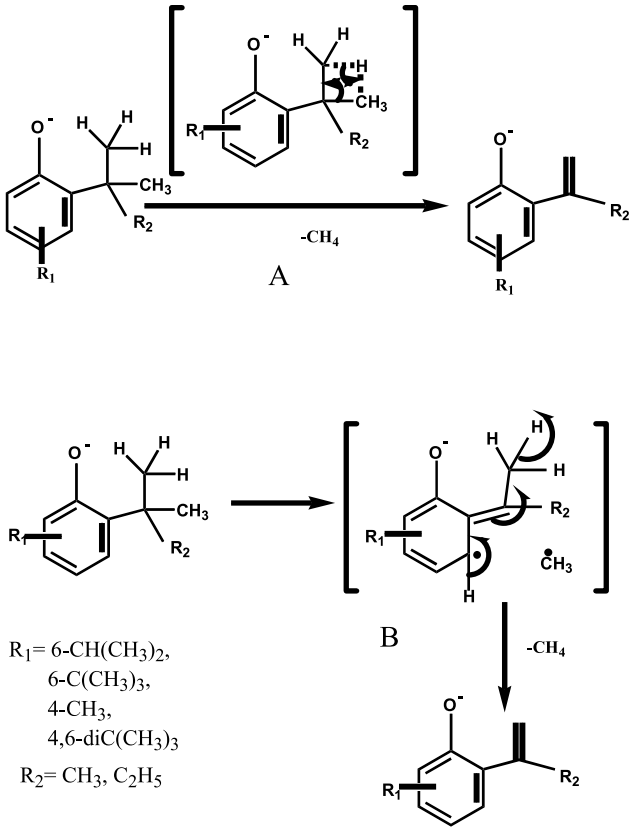


Figure 3. High resolution product ion spectrum of *m/z* 135, the $[M - H]^-$ ion of 2-isopropyl phenol. There are two distinct product ions at *m/z* 119 and 120.



Scheme 3

m/z 16. In the case of secondary butyl and tertiary amyl phenols (sample numbers 12–14), which have a methyl as well as an ethyl group in the β position, the most prominent product ion was formed by the loss of *m/z* 30, which may correspond to a loss of ethane rather than methane.

Table 1 also shows indirect evidence for the proposed loss of methane as opposed to oxygen from the initial phenol. As could be expected from the methyl and the ethyl substituted phenols (sample numbers 21–23 and 15, respectively), which have no β methyl group, daughter ions were formed by the loss of *m/z* 15, which could be attributed to a methyl group. Similarly, catechol (sample number 20) did not show loss of *m/z* 16. These findings support the contention that the loss of *m/z* 16 does not correspond to a loss of an oxygen atom from the phenol, because oxygen was present in all these phenols, whereas a methyl group in the β position of the substituent was not. The proposed loss of methane was confirmed by doing further product ion scans of 2-isopropyl phenol.

Figure 2 shows the product ion spectra of the $[M - H]^-$ ion of 2-isopropyl phenol. If the loss of *m/z* 16 from isopropyl phenol's $[M - H]^-$ ion corresponds to an oxygen atom, then further fragmentation of the *m/z* 119 product ion should lead to a product ion at *m/z* 91 because of the loss of C_2H_4 (Scheme **2**). Conversely, if the loss of *m/z* 16 from the $[M - H]^-$ ion corresponds to the loss of methane, further fragmentation of the product ion should produce product ions of *m/z* 93 (loss of C_2H_2), which in turn could lose CO (nominal mass 28) to give rise to a product ion at *m/z* 65. The proposed

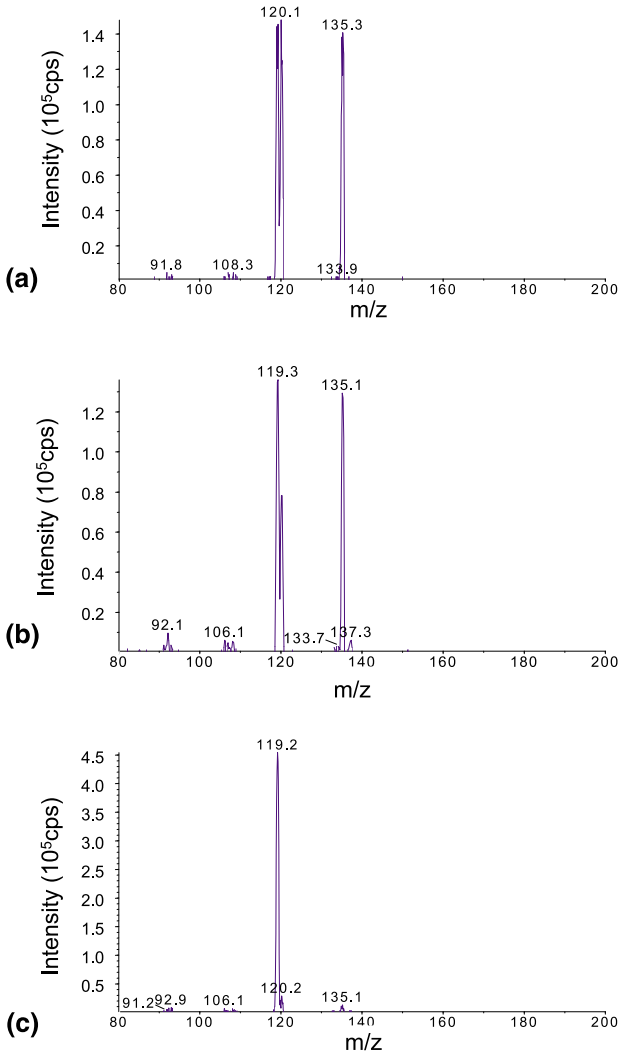
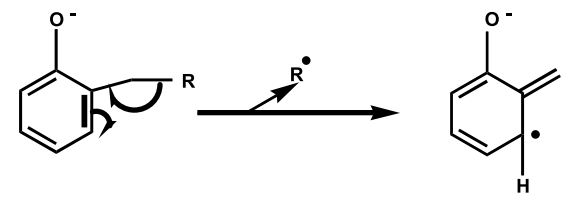


Figure 4. Product ion spectrum of the $[M - H]^-$ ion of 3-isopropyl phenol at increasing levels of collision-induced dissociation (**a**) collision energy 25 eV and collision gas supply pressure 2 psi; (**b**) collision energy 25 eV and collision gas supply pressure 6 psi; (**c**) collision energy 30 eV and collision gas supply pressure 6 psi.



Scheme 4

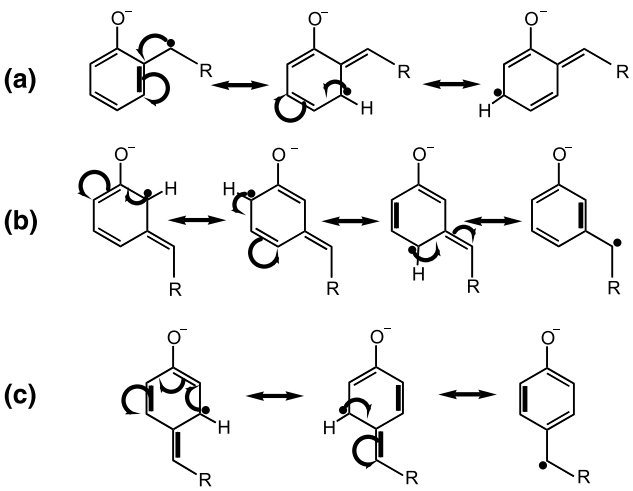
Table 2. Intensities of the most abundant product ions $[M\text{-}H\text{-}CH_4]^-$ of ortho, meta, and para substituted phenols with APCI in negative ion mode

Phenol	Parent ion $[M\text{-}H]^-$ (*m/z*)	Collision energy (eV)	Most abundant product ion (*m/z*)	Intensity of parent ion (10^4 cps)	Intensity of product ion (10^4 cps)	Intensity ratio product/ parent
2-Isopropyl phenol	135.0	35	118.9	2300	4	0.002
3-Isopropyl phenol	135.0	35	119.1	2400	280	0.117
2-Tertiary butyl phenol	149.0	35	133.1	630	15	0.024
3-Tertiary butyl phenol	149.0	35	133.1	2600	390	0.15
4-Tertiary butyl phenol	149.0	35	133.1	2000	28	0.014
2,6-Ditertiary butyl phenol	205.1	35	189.2	3000	20	0.007
3,5-Ditertiary butyl phenol	205.0	35	189.2	3400	400	0.118

structure for *m/z* 119 was further supported by comparing its product ions with the product ions of 4-vinyl phenol, fragmented under identical conditions (data not shown). These experiments also produced daughter ions at *m/z* 93 and 65, two common fragmentation peaks for the $[M^\circ - H]^-$ ions of most phenols [22]. As is evident from Figure 2, there were no product ions corresponding to *m/z* 103, 91, or 77, whereas there is a product ion at *m/z* 93. Taken together, these findings support the assumption of a loss of methane from the initial phenols.

Further evidence for the proposed loss of 16 as a methane molecule, as opposed to an oxygen atom, was provided by recording high resolution product ion mass spectra of the $[M - H]^-$ ion of 2-isopropyl phenol [*m/z* 135.0829 (14.1 ppm error)] (Figure 3). Precise determination of the mass of the product ion at *m/z* 119 yields a mass of 119.0481 (13.4 ppm error). This measurement supports the ionic formula of $C_8H_7O^-$ formed by the loss of methane from the $[M - H]^-$ ion of 2-isopropyl phenol with a calculated mass of 119.0497 (Δ = 0.0016, error of 13.4 ppm), rather than $C_9H_{11}^-$ formed by the loss of oxygen with a calculated mass of 119.0861 (Δ = 0.0380, error of 319 ppm). Furthermore, the product ion attributable to mass loss of 15 at *m/z* 120.0550 (20.8 ppm) can not be explained by the loss of a single oxygen atom, but is entirely consistent with the intermediary fragmentation product based on the two-step fragmentation mechanism for the loss of methane discussed below.

Two possible mechanisms for the loss of *m/z* 16 upon CID of the $[M - H]^-$ ion of various substituted phenols (Table 1, sample numbers 1–11) are shown in Scheme **3**. One is a simple concerted four-centered elimination of methane (Scheme **3a**) and the other is a stepwise elimination involving an initial cleavage to form an intermediate ion-molecule complex (Scheme **3b**) and a subsequent proton transfer within the complex, which is similar to the mechanisms reported for the elimination of methane from alkoxide ions in the gas phase [19–21]. Consistent with the stepwise fragmentation mechanism, product ions corresponding to the intermediate radical ion B were seen for almost all phenols when product spectra were acquired at lower collision energies of 20 to 25 eV. Such intermediate product ions can not be explained by the concerted mechanism (Scheme **3a**). However, in none of these cases was it possible to observe the ion radical B as the major product ion. For example, in the case of 3-isopropyl phenol (Figure 4), the product ion arising from the loss of a methyl radical at *m/z* 120 was formed along with the product ion at *m/z* 119 in equal intensity when the spectrum was recorded at a collision energy of 25 eV and a collision gas supply pressure of 2 psi (Figure 4a). An increase in the collision gas supply pressure from 2 to 6 psi at the same collision energy increased the intensity of the product ion at *m/z* 119 at the expense of the product ion at *m/z* 120 (Figure 4b). As the collision energy increased to 30 eV the product ion at *m/z* 119 became the major ion peak (Figure 4c). Encouraged by this observation, we presumed that fragmentation of a phenol with only one substitution at the β position would allow us to observe an ion radical similar to this intermediate ion radical at *m/z* 120 as the predominant ion peak, because there will be no β hydrogen available for further loss of an H radical. With this strategy, CID of the $[M - H]^-$ ion of n-ethyl, n-propyl, n-butyl, n-pentyl, and n-heptyl phenols was attempted. The product ion spectra showed, as expected, *m/z* 106 as the



Scheme 5

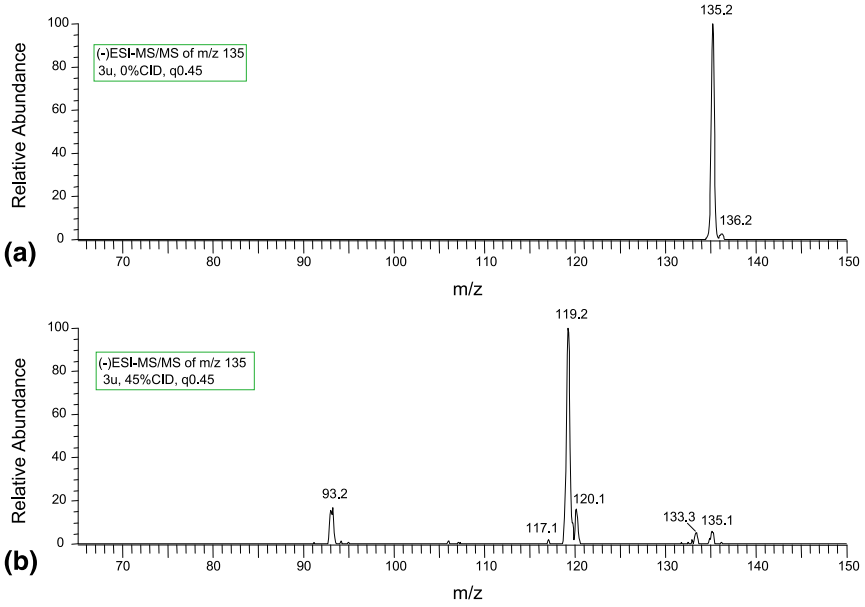


Figure 5. Mass spectral fragmentation of the $[M - H]^-$ ion of 2-isopropyl phenol using electrospray ionization in negative ion mode. The product ions are the same as with APCI shown in Figure 2. (**a**) Normal mass spectrum; (**b**) product ion spectrum of *m/z* 135 at 45% CID. These spectra were recorded with a Finnigan LCQ mass spectrometer.

only major product ion (Table 1, sample numbers 15–19). The fragmentation process is shown in Scheme **4**.

The two-step mechanism for the loss of methane by the sequential loss of CH_3 and H radicals was further supported by comparing the abundances of product ions formed from different ortho, meta, and para substituted phenols. Table 2 shows the intensities of the product ions formed by the loss of methane from the $[M - H]^-$ ion of phenols substituted at ortho, meta, and para positions with the same substituents. Compared to product ions from ortho and para substituted phenols, those from meta substituted phenols were more stable and always formed in greater relative abundance (typically 20-fold more intense) irrespective of the type of substituent present. This finding is consistent with the proposed mechanism for the fragmentation shown in Scheme **3** because the intermediate radical ions resulting from meta substituted phenols will have more resonance structures (Scheme **5**) and are therefore more likely to form and are more stable.

CID of the $[M - H]^-$ ion of each of these phenols was also performed after ionization with ESI in the negative ion mode. For all phenols, the pattern of fragmentation was almost the same as after ionization with APCI. Figure 5 shows product ion spectra of the $[M - H]^-$ ion obtained from ESI of 2-isopropyl phenol. The product ions are identical to those obtained in APCI MS/MS shown in Figure 2. These results further support the fragmentation pathways for the $[M - H]^-$ ion of simple substituted phenols depicted in Schemes **3** and **4**. APCI was selected over ESI as the source of choice for the LC/MS/MS assay for propofol for three major reasons. First, APCI is a more predictable and robust ion source for small compounds that are thermally stable and volatile. Second, APCI is more compatible with high HPLC flow rates than ESI. Finally, ESI sensitivity is greatest at low flow rates but it can suffer from unreliable detection efficiency for small drug compounds [23].

Conclusions

The mass spectral fragmentation of the $[M - H]^-$ ion of propofol involves the sequential loss of a methyl radical and a hydrogen radical in a two-step process to give the most intense product ion at *m/z* 161. This two-step process was also the preferred mode of fragmentation for the $[M - H]^-$ ion of structurally similar phenols with branched alkyl substituents. Phenols with a straight chain alkyl substituent, in contrast, underwent β elimination of the alkyl radical irrespective of the length of the alkyl chain, yielding the most intense product ion at *m/z* 106. This product ion represents a special case of a stable radical for the two-step process described for branched side chains because further elimination of a hydrogen radical from the β carbon is not possible.

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