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Evaluation and Optimization of APGC Parameters for the Analysis of Selected Hop Essential Oil Volatiles

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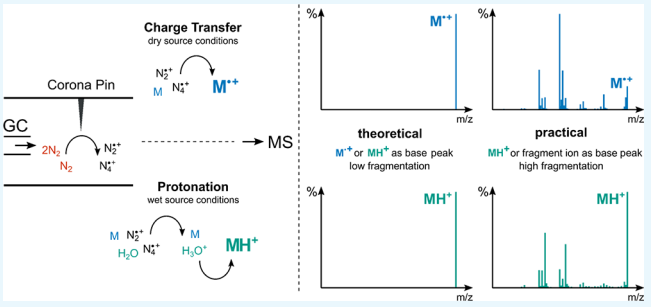
ABSTRACT: Hop essential oil is a mixture of several hundred volatile metabolites that quantitatively and qualitatively distinguish hop varieties. Given the commercial relevance of hops in the brewing industry and the complexity of hop oil, analytical tools enabling a comprehensive characterization of oil constituents are required. At this, atmospheric pressure chemical ionization interfaced to gas chromatography and high-resolution mass spectrometry (APGC−MS) is a promising option that combines soft ionization, high sensitivity, and high resolution. While high sensitivity is required to detect minor or trace-level volatile metabolites, soft ionization and high resolution enable the reliable identification of unknowns based on exact masses of the molecular ion or the protonated molecule. Twenty-two volatile metabolites typically found in hop oil were studied in respect to their APGC ionization behavior. For 15 compounds, APGC−MS did not yield high molecular ion or protonated molecule intensities and considerable in-source fragmentation was observed. APGC−MS parameter optimization (cone gas flow and cone voltage) was able to yield the maximum absolute intensity for the base peak. However, in-source fragmentation could not be prevented, leading to spectra with either the protonated molecule or a characteristic fragment ion as the base peak. APGC−MS operated under optimized parameters was applied to a hop essential oil sample to verify the effect of optimization. By estimating the limit of quantification for the 22 compounds, it is concluded that APGC−MS is well suited to analyze major, minor, and trace-level volatiles from hops.



INTRODUCTION

Among the ingredients used for brewing, hops are the one used to create beer bitterness and aroma. While beer bitterness primarily results from a series of chemically similar, non-volatile bitter acids (humulones, iso-humulones, and humulinones), hop aroma in beer results from an enrichment of volatile hop secondary metabolites during brewing.[1] The volatile metabolites of hops are commonly referred to as hop essential oil and are extracted by steam distillation. Hop essential oil contains a great variety of terpenes, terpenoids, esters, ketones, aldehydes, and acids as well as several trace-level sulfur compounds[2,3] and represents approx. 1−2.5% of hop dry matter. The total concentration and composition of hop essential oil depend on multiple factors, most importantly plant genetics (hop variety) but also geography (growing region) and harvest maturity.[4] Industrial beer production primarily uses hop pellets or extracts; thus, hop processing and associated handling (drying, milling, pelletizing, extraction, storage, etc.) might also impact hop essential oil concentration and composition. More than 250 hop varieties are currently used in the brewing industry[5] and are selected based on their characteristic aroma.[1]

To analyze hop aroma, several approaches exist. These range from simple, gas chromatography−flame ionization detector (GC−FID) methods[6,7] to advanced GC−electron impact ionization−mass spectrometry (GC−EI−MS) based assays with automated sample preparation.[1] To comprehensively study hop essential oil composition, untargeted metabolomics, using GC−HRMS instrumentation, appears suitable to identify (unknown) variety discriminating hop volatile metabolites. More recently, GC × GC and GC−GC × GC coupled to high-resolution mass spectrometry (HRMS) were used to improve the chromatographic separation of complex hop essential oil samples and to acquire exact masses.[8−10] When HRMS is interfaced to GC, the ionization technique most commonly used is EI. Employing EI results in extensive fragmentation and highly reproducible mass spectra, which is a major benefit when, for example, aiming to quantify compounds in complex mixtures. This is a major benefit for advanced (trace-level) quantification methods.

When aiming to identify unknowns, a GC−MS/MS setup is ideal. Selecting the molecular ion as a precursor ion yielding

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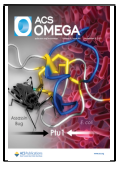

fragment ions provides equally important information in terms of the elemental and structural composition. As the relative intensity of the molecular ion is frequently low using EI, other ionization methods promoting the detection of the molecular ion in high relative intensities seem beneficial.

Atmospheric pressure chemical ionization (APCI), first described by Horning et al. in 1973,[11] is a "soft" ionization technique and is frequently used in liquid chromatography (LC)−MS applications targeting nonpolar molecules. Horning et al. demonstrated that coupling of APCI to GC (APGC) is possible (Figure 1a). Only in the last 20 years, major instrument suppliers (Waters Corporation, Bruker, Thermo Fisher, and Agilent) have developed commercially available APGC sources, offering usage of one HRMS analyzer by LC and GC. This allows the coverage of volatile and nonvolatile analytes on a single platform. Especially for food research, this combination is of considerable interest, as flavor is not only defined by the volatile fraction of a sample.[12,13] As ionization with APGC has been shown to result in mass spectra characterized by abundant molecular ions and/or protonated molecules and little fragmentation,[14−16] allowing the selection of high m/z ions for further MS/MS experiments, APGC−MS has been applied to various fields of research such as food,[17,18] metabolomics,[19−23] pesticides,[16,24] or steroids.[25]

Ionization with APGC is an indirect ionization enabling two ionization mechanisms: charge transfer and protonation (Figure 1b). The initial step is the ionization of the makeup gas (nitrogen) at the corona pin. For charge transfer under dry source conditions (no water present in the ion source), analyte molecules are subsequently ionized, resulting in radical cations ($[M^{\bullet}]^+$). For protonation under wet source conditions (water is present in the ion source), water molecules are first ionized, yielding oxonium ions (H_3O^+) that then ionize the analyte molecules via proton transfer resulting in cations ($[M + H]^+$).[26] The preferred ionization mechanism depends on the analyte structure[27] and source conditions.

Based on the fact that the comprehensive analysis of hop essential oil is challenging but of significant relevance for the brewing industry, powerful analysis tools are needed. APGC interfaced to quadrupole-ToF (APGC−Q-ToF) is a promising technique for both targeted and untargeted metabolomics analyses. Hence, the current study aimed to (1) evaluate the APGC ionization behavior of 22 representative hop aroma compounds; (2) optimize source conditions to retain molecular ions, protonated molecules, or characteristic fragment ions; and (3) estimate benefits and challenges associated to the use of APGC−Q-ToF in targeted and untargeted metabolomics of hop and potentially other plant essential oils.

RESULTS AND DISCUSSION

APGC Results in High In-Source Fragmentation for All Analytes and Low Intensities of Molecular Ions or Protonated Molecules for Terpenoids and Esters. The standard mix was analyzed using dry and wet source conditions (Table 1) to assess the predominant ionization mechanism. Resulting spectra were screened for the theoretical masses of the molecular ions and protonated molecules, $[M^{\bullet}]^+$ and $[M + H]^+$, of the analytes (Table S1). Using APGC default parameters resulted in at least either one or both the molecular ion or protonated molecule under both source conditions, except for the four isobutyl esters and 2-methylbutyl 2-/3-methylbutyrate, where no molecular ion or protonated molecule was observed (Figure S1). Although all analytes were

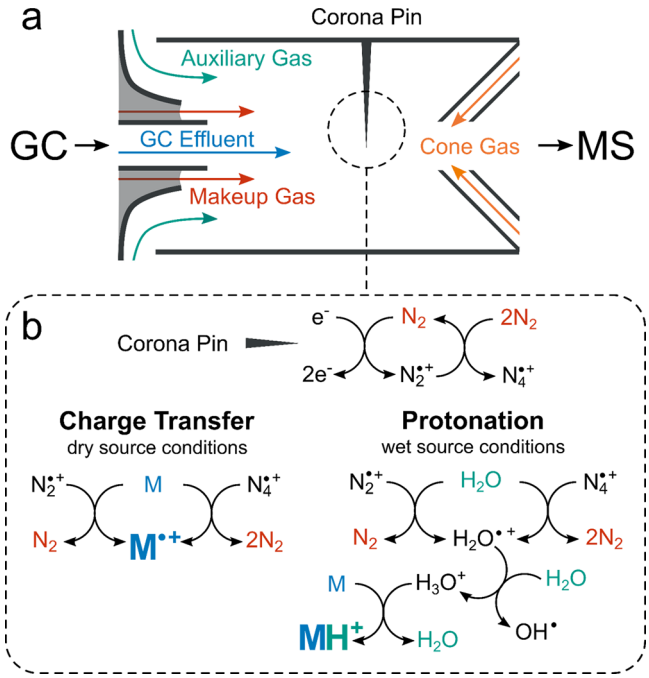

Figure 1. (a) Schematic representation of the APGC ion source (adapted from ref 28). (b) APGC ionization mechanisms.[26]

Table 1. APGC Source Parameters Used for Acquisition under Default Parameters during Optimization and for the Optimized Method

acquisition	auxiliary gas [L/h]	corona current [μA]	cone gas [L/h]	cone voltage [V]
default parameters	220	2.2	120	30
variation of cone gas	300	2	80−200	20
variation of cone voltage	300	2	100	5−30
optimized method	300	2	100	5

injected in equal concentrations, the absolute intensities of their $[M + H]^+$ and $[M^{\bullet}]^+$ ions differed strongly, if present. The highest absolute intensities were detected under wet source conditions for the $[M + H]^+$ ion of α-humulene (~3.5e7), β-caryophyllene (~2.5e7), and methyl 2-methylbutyrate (~2.3e7) (Table S2).

To benchmark the ionization behavior, spectra recorded under APGC conditions of the analytes were compared to EI reference spectra obtained from NIST. To improve readability, exact masses acquired by APGC−Q-ToF are written as nominal masses. It must also be noted that the mass range of APGC−Q-ToF was m/z 50−650, and ions $<m/z$ 50 are therefore only displayed within the EI reference spectra.

Depending on their chemical structure, the ionization behavior of the analytes under APGC default parameters varied and can thus be categorized into four groups. Subsequently, only four analytes are discussed: myrcene (representing (−)-β-pinene, (+)-limonene, α-humulene, β-caryophyllene, 2-undecanone, and methyl 2-methylbutyrate), linalool (representing (+)-α-terpineol, *cis*-linalooloxide, and (−)-caryophyllenoxide), geranyl acetate (representing methyl geranate, geranyl propionate, and geranyl isobutyrate), and 2-methylbutyl isobutyrate (representing 3-methylbutyl iso-

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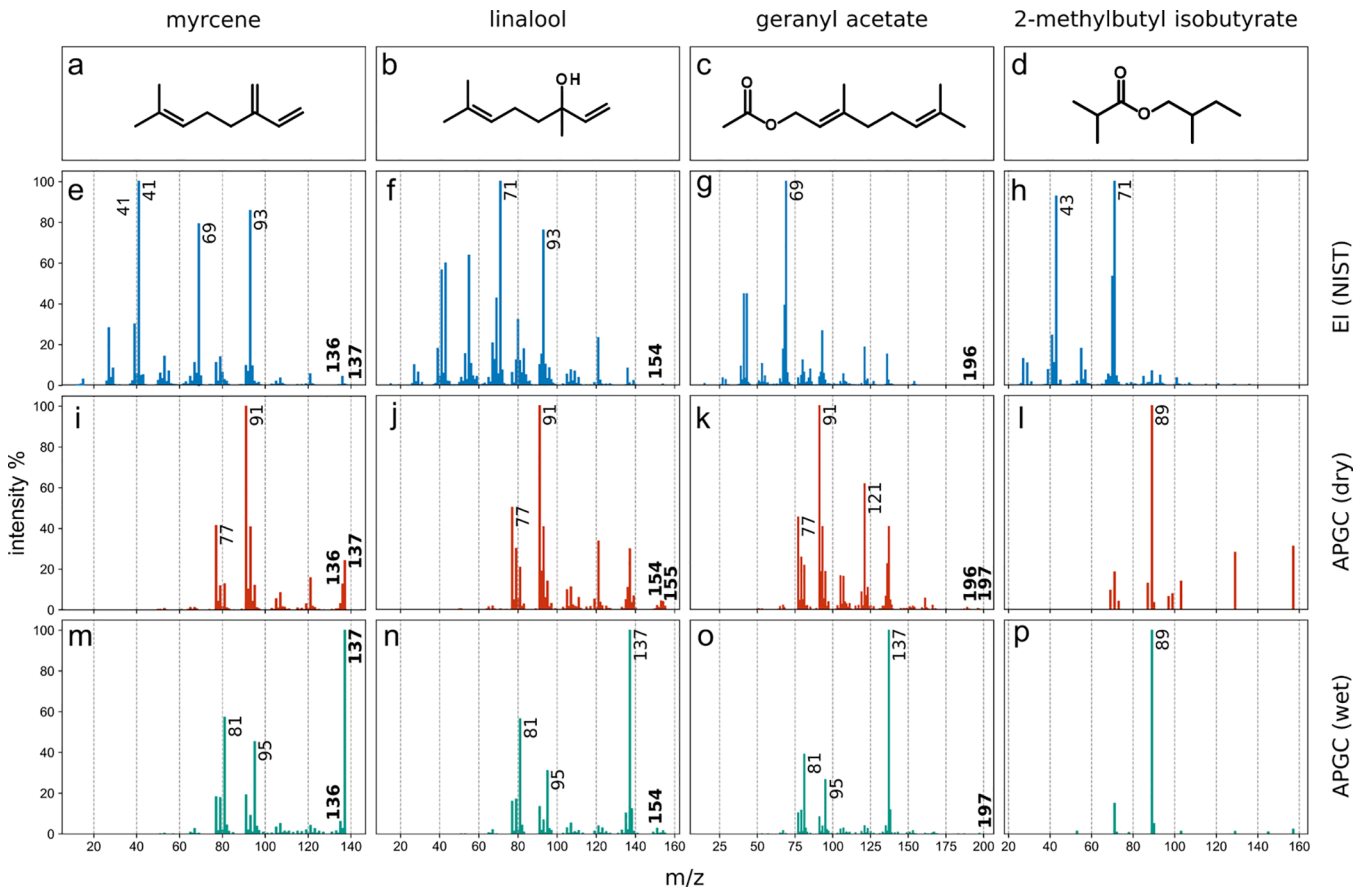

Figure 2. Molecular structures, EI spectra, and spectra recorded under APGC conditions for myrcene, linalool, geranyl acetate, and 2-methylbutyl isobutyrate. (a–d) Molecular structures; (e–h) EI spectra obtained from NIST; (i–l) spectra recorded under APGC dry source conditions; and (m–p) spectra recorded under APGC wet source conditions. Due to the mass range of APGC–Q-ToF (m/z 50–650), ions <m/z 50 are only displayed within EI spectra. Masses of the molecular ions or protonated molecules are depicted in bold numbers.

butyrate, 2-methylbutyl 2-methylbutyrate, 2-methylbutyl 3-methylbutyrate, isobutyl isobutyrate, butyl isobutyrate, and propyl 2-methylbutyrate) (Figure 2). Data for all other analytes are available in the Supporting Information (Figures S2–S4 and Table S2).

The EI reference spectra of myrcene, linalool, geranyl acetate, and 2-methylbutyl isobutyrate are characterized by extensive fragmentation; the presence of one (geranyl acetate) to three (myrcene) abundant fragments (Figure 2e–h); and, if present, low relative intensities of the molecular ion or protonated molecule (~0.1–7%) (Table S2). Unexpectedly, opposed to the assumption that spectra recorded under APGC conditions are dominated by the molecular or protonated ion, the acquired low-energy spectra under dry and wet source conditions presented considerable in-source fragmentation (Figure 2i–p). Under APGC dry source conditions, myrcene, linalool, and geranyl acetate presented both the $[M^{\bullet}]^+$ and $[M + H]^+$ ions, with relative intensities of 0.2–24% (Table S2), whereas 2-methylbutyl isobutyrate presented no molecular ion or protonated molecule (Figure 2i–l). Myrcene, linalool, and geranyl acetate presented a similar fragmentation pattern (Figure 2i–k), with the fragment ion m/z 91 as the base peak. This was also true for most of the other analytes within the respective groups, except for 2-undecanone, methyl 2-methylbutyrate, (−)-caryophyllenoxide, and methyl geranate (Figures S2 and S3). 2-Methylbutyl isobutyrate (Figure 2l) and all other esters in its group presented a less extensive fragmentation pattern with one distinctive fragment ion as base peak. 2-Methylbutyl isobutyrate showed a base peak at m/z 89, which was found to be characteristic for isobutyric esters, while 2-/3-methylbutyric esters presented a characteristic base peak at m/z 103 (Figure S4). Within the spectra recorded under APGC wet source conditions for myrcene, linalool, and geranyl acetate (Figure 2m–o), the in-source fragmentation was less distinct compared to that under dry source conditions. Abundant fragment ions detected under wet source conditions differed from those detected under dry source conditions (Figure 2i–l). The $[M + H]^+$ ion was the base peak for myrcene, while the base peak for linalool and geranyl acetate shifted to a fragment ion with higher a m/z (m/z 137). The relative intensity of the molecular ions or protonated molecules for linalool and geranyl acetate did not change significantly (Table S2). For 2-methylbutyl isobutyrate, wet source conditions did not change the fragmentation pattern compared to dry source conditions (Figure 2l–p), nor did they increase the relative intensity of the molecular ion or protonated molecule (Table S2). A possible explanation is that molecules with functional groups are more prone to in-source fragmentation.

To evaluate APGC performance, it was of particular interest to estimate to which extent the ratio of the molecular ion or protonated molecule to the highest intensity fragment (M/F) changed from EI to APGC (Table S2). Only for the $[M + H]^+$ ions of myrcene was a distinct increase of the M/F% from EI

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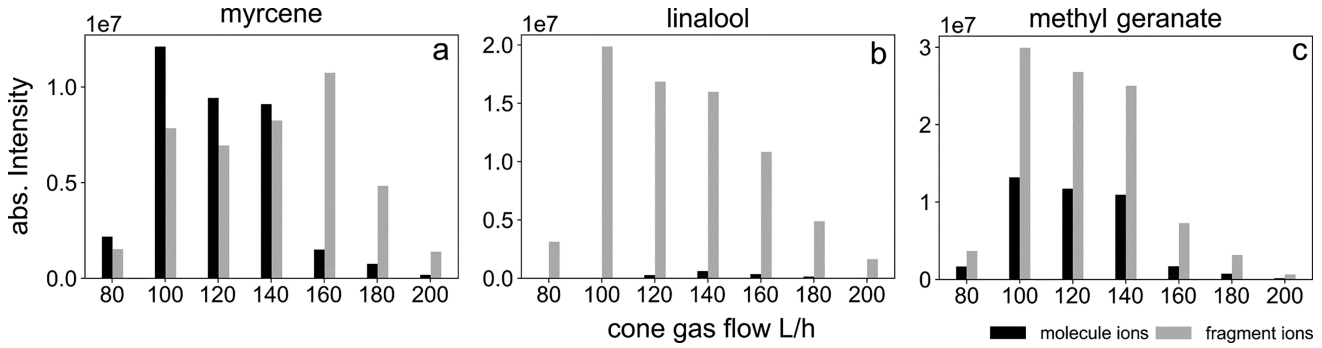

Figure 3. Sum of the absolute intensities of the molecular ions and protonated molecules and the fragment ions (highest intensity fragment ions at cone gas flow values of 80 and 200 L/h, respectively; Table S1) over the cone gas flow optimization range. (a) Myrcene, (b) linalool, and (c) methyl geranate.

(0.5%) to APGC dry conditions (25%) to wet conditions(175%) observed. This trend was also observed for the analytes in its group and methyl geranate. For (−)-caryophyllenoxide, the M/F% increased under dry source conditions (51%) and decreased under wet source conditions (27%). For all other analytes, the M/F% did not differ significantly. Further, absolute intensities of the base peak increased from dry to wet APGC source conditions for 80% of all analytes (Table S2). These results lead to the conclusion that under wet source conditions protonation was overall the most efficient ionization mode, although in-source fragmentation could not be prevented. Myrcene showed high intensity fragments at *m/z* 91 (APGC dry), *m/z* 93 (EI), and *m/z* 95 (APGC wet) (Figure 2e,i,m). Presumably, APGC generates the well-observed terpene EI fragment at *m/z* 93, which is likely to occur by cleavage at one of the molecules' quaternary centers.[29,30] While APGC under dry source conditions causes the loss of two hydrogen atoms (*m/z* 91) from the EI fragment, under wet conditions, it causes the addition of two hydrogen atoms (*m/z* 95). The base peak for linalool (*m/z* 137) presumably corresponds to a loss of water from the protonated molecule, which essentially generates a protonated terpene molecule (Figure 2b). These findings are in accordance with the results of a study analyzing terpene and terpene alcohols with proton transfer reaction−MS (PTR−MS). PTR−MS is similar to APGC−MS under wet source conditions, as H_3O^+ acts as the ionizing agent of the analyte molecules.[31] Geranyl acetate also presented a base peak at *m/z* 137, corresponding to an elimination of the acid entity of the ester, leaving a protonated terpene (Figure 2c).[32] For 2-methylbutyl isobutyrate, APGC under both source conditions yielded a characteristic fragment, indicating the elimination of the alcohol group with the migration of one hydrogen atom (Figure 2d).[32]

In summary, these results show that analytes such as terpene alcohols, terpene alcohol oxides, and sesquiterpene oxides as well as aliphatic/terpenoic esters are unstable under APGC default parameters, which result in characteristic in-source fragmentation. On the contrary, terpenes and sesquiterpenes that feature no functional groups, but also 2-undecanone and methyl-2-methylbutyrate were less prone to in-source fragmentation yielding a dominant protonated molecular ion under APGC default wet conditions.

Other studies focusing on APGC ionization used derivatization with BSTFA and/or MSTFA on analytes such as amino acids, phenolic acids, glycolysis intermediates, flavonoids, fatty acids, and anabolic androgenic steroids.[17,19−21,23,25] In all studies, the protonated derivatized analyte was considered as the $[M + H]^+$ ion and minimal in-source fragmentation was reported for most of the analytes. However, the fragment ions formed corresponded to a loss of a derivatization group, demonstrating that derivatization acts as a stabilizer and suppresses in-source fragmentation of the actual analyte.

Although derivatization is a straightforward solution to increase the volatility and thermostabilty of polar metabolites, which also reduces APGC in-souce fragmentation, it is not generally feasible when aiming for coverage of the volatile metabolome of hops. First, hop oil, as mentioned in the introduction, contains volatiles of many chemical classes so that no single derivatization method is sufficient. Second, none of the common derivatization methods (silylation, acylation, and alkylation) is able to derivatize analytes, such as esters, that are highly prone to in-source fragmentation. Third, derivatization of acids, alcohols, and thiols may have different requirements regarding the concentration, temperature, and time, resulting in a laborious sample preparation.

Formation of the Molecular Ion or Protonated Molecule and In-Source Fragmentation Depend on Substance Class and Not on APGC Parameters. Molecular ions or protonated molecules provide important information when aiming for identification of unknowns. To cover the volatile metabolome of hops without derivatization, reduction of in-source fragmentation was attempted by optimizing APGC source parameters (Table 1). As stated by the instrument manufacturer, cone gas flow is the essential parameter to control ionization mechanism and optimize ionization efficiency. Cone gas acts as a counter stream to the auxiliary gas, the makeup gas, and the GC effluent within the source (Figure 1a). For proton transfer (wet source conditions), H_2O should be drawn into the source by the auxiliary gas; thus, low cone gas flow values are used. On the contrary, charge transfer (dry source conditions) is promoted using high cone gas flow values, as ideally no water enters the source. Furthermore, high cone gas flow values cause less ions to enter the sample cone, thereby causing lower absolute intensities.[28] In-source fragmentation caused by high cone voltages has been described for ESI, caused by the collisional activation of the ions passing from the atmospheric pressure regions of the source to the vacuum region of the mass analyzer.[33] Several studies report in-source fragmentation using APGC caused by high cone voltages.[18,34−36] APGC default wet source conditions generated spectra with overall higher absolute intensities for all ions and fewer high relative intensity fragment ions compared to default dry conditions; thus, wet

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source conditions were maintained during ionization optimization. To promote protonation, auxiliary gas flow was kept at high values (300 L/h). Cone gas flow values in the range of 80−200 L/h were applied to survey if low absolute and relative intensities of the molecular ion or protonated molecule were due to unfavorable cone gas flow settings. After evaluating the cone gas values resulting in the highest ion intensities, cone voltage values were set to 5−30 V to investigate the in-source fragmentation behavior of the analytes (Table 1).

To monitor changes in ionization mechanism and efficiency, for all analytes, the sum of the absolute intensities of both the molecular ion and protonated molecule was compared to the sum of the absolute intensities of the fragment ions with the highest relative intensity at 80 and 200 L/h, representing the characteristic fragment ion at protonation or charge transfer conditions (Tables S1 and S2). As in the previous section, analytes were grouped based on their ionization behavior during optimization. The myrcene group remained unchanged, and methyl geranate and (−)-caryophyllenoxide built a new group. All other analytes are now represented by linalool. Thus, only myrcene, linalool, and methyl geranate are discussed further. Data for all other analytes are available in the Supporting Information (Figure S5 and Table S2).

At low cone gas flows (80−140 L/h), the molecular ion and protonated molecule for myrcene had a higher intensity than the fragment ions. This relation was inverted for high cone gas values (160−200 L/h) (Figure 3a). For linalool, the molecular ion and protonated molecule are not detected or occur in very low intensities over the complete cone gas flow optimization range, resulting in the fragment ions having the highest intensities (Figure 3b). Methyl geranate presented the molecular ion and protonated molecule for low cone gas flows (80−140 L/h), but the fragment ions had a higher intensity over the complete cone gas flow optimization range (Figure 3c). Over the complete range of cone gas flow values, maximum absolute intensities were found at 100 L/h for either the protonated molecule or a fragment ion (Table S2). In-source fragmentation could not be prevented and was predominant at high cone gas values (160−200 L/h). Thus, a cone gas flow of 100 L/h was chosen as the optimal value and used for the optimization of the cone voltage.

To monitor cone voltage associated changes in the in-source fragmentation, cone voltage was varied (5−30 V), while cone gas flow was kept at the previously optimized value of 100 L/h. To assess changes in in-source fragmentation, both the molecular ion and protonated molecule and the fragment ions with the highest relative intensity at cone gas flows of 80 and 200 L/h were monitored for all analytes, as for the optimization of the cone gas flow (Table S1). As opposed to the expectation that lower cone voltage values result in little or no fragmentation, neither a change in fragmentation nor an increase of the molecular ion or protonated molecule could be observed (Table S2). Overall, absolute intensities were the highest for $[M + H]^+$ and/or the fragment ion corresponding to a cone gas flow of 80 L/h at a cone voltage value of 5 V. As the above-stated hypothesis of less in-source fragmentation at lower cone voltage values might be true for other hop essential oil constituents not included in the standard mix, a final cone voltage of 5 V was chosen as the optimal value.

These results show that in-source fragmentation is dependent not on the APGC parameters cone gas flow and cone voltage but on the chemical structure of the analytes.

Optimized APGC Parameters Are Suitable for Hop Essential Oil Analysis. With the prospect of APGC−Q-TOF being suitable for untargeted metabolomics, a hop essential oil sample was subjected to analysis with default parameters under dry and wet source conditions and optimized parameters. To assess the changes in ionization, histograms of the detected ions over a retention time range of 5−34 min were created. While the binning was based ion *m/z*, the respective absolute intensities were used as weights for the frequency. Comparing default dry to wet source conditions (Figure 4a) shows a shift

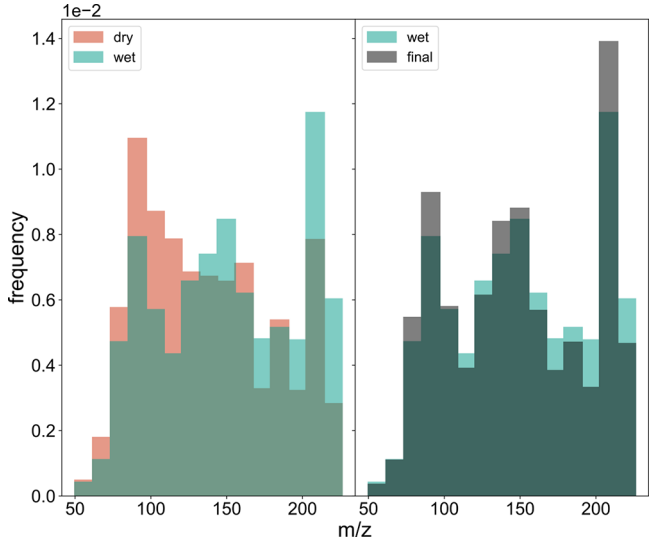

Figure 4. Histograms of detected ions (R_T: 5−34 min) of a hop essential oil sample under default dry and wet source conditions and optimized parameters. Comparison of default dry and wet source conditions (a) and default wet source conditions and optimized parameters (b).

in the frequency from lower to higher *m/z* values (*m/z* ∼90 to ∼140 and ∼200). This observation likely relates to a promotion of the formation of the molecular ion or protonated molecule of the analytes and less dominant in-source fragmentation. This is in accordance with the observations made by acquisition of APGC data under dry and wet source conditions (Figure 2). Comparing default wet and optimized parameters, the distribution within the histograms is highly comparable, but the absolute intensities increased, represented by an increase in the frequency (Figure 4b). Frequency maxima for optimized parameters can be seen at *m/z* ∼90−110, 130−150, and 200−210, corresponding to protonated molecules or fragment ions of esters (*m/z* 89 and 103), terpenes and terpene alcohols (*m/z* 137), and sesquiterpenes (*m/z* 205) (Table S2).

Thus, optimized source conditions had a positive effect on the detectable analytes in the hop essential oil sample. Still, in-source fragmentation could not be prevented by the optimization of APGC parameters (Figure S6). This surely represents an obstacle using an untargeted metabolomics workflow. If no in-source fragmentation would occur using APGC, identification could be performed based on molecular ions or protonated molecules and retention time. However, with in-source fragmentation, identification is not trivial. For any detected signal, it is not clear whether it results from a molecular or fragment ion. Taking *m/z* 137 as an example, this might be the protonated molecule of a terpene, a fragment ion

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of a terpene alcohol, or even a terpene alcohol oxide (Figure 2). To use APGC−Q-ToF data of hop oils for untargeted metabolomics, a computational approach based on substance group characteristic fragments, their ratio, and retention time is needed for data processing. However, a major advantage is the sensitivity of the instrument. Based on absolute intensities of the analytes in the standard mix, acquired with the optimized APGC method, and the assumption of a hop sample yield of 1.5 mL hop essential oil/100 g hops, the limit of quantification was estimated to be well below <1 μg/kg for all analytes (Table S3). Based on this finding, one can conclude that APGC sensitivity enables the analysis of major, minor, and trace-level hop essential oil components.[37]

CONCLUSIONS

This study investigated the APGC ionization behavior of 22 representative hop aroma compounds. It was found that 15 of the 22 compounds studied underwent considerable in-source fragmentation and did not, as expected, yield high molecular ion or protonated molecule intensities. While the modification of the source conditions was able to increase the yield of the protonated molecule for terpenes, sesquiterpenes, and ketones, this was not the case for terpenoids and esters. Still, for the latter substance groups, characteristic fragment ions could be optimized regarding their absolute intensities.

Overall, optimal APGC source parameters were found to be wet source conditions promoting the protonation of the analytes with low cone gas flow (100 L/h) and low cone voltage (5 V). Optimized parameters were validated by applying default and optimized APGC parameters to a hop essential oil sample, resulting in high intensity ions with higher m/z representing molecular ions, protonated molecules, or characteristic high m/z fragment ions. Analysis of the hop essential oil sample further showed that much information can be retained from APGC−Q-ToF analysis of hop oil, calling for an untargeted metabolomics application. As in-source fragmentation could not be prevented, one major drawback is the uncertainty if an ion is in fact a molecular ion/protonated molecule or a fragment ion. Computational procedures can be implemented to overcome this obstacle, leading the way for further research. In summary, APGC−Q-ToF seems to be a suitable tool for targeted and untargeted analysis of major components of hop and other essential oils.

EXPERIMENTAL SECTION

Standards and Chemicals. Analytical standards of myrcene, (+)-limonene, (−)-β-pinene, α-humulene, β-caryophyllene, linalool, *cis*-linalooloxide, (−)-caryophyllenoxide, methyl 2-methylbutyrate, butyl isobutyrate, 3-methylbutyl isobutyrate, 2-methylbutyl 3-methylbutyrate, 2-methylbutyl 2-methylbutyrate, geranyl isobutyrate, geranyl propionate, and geranyl acetate were purchased from Sigma-Aldrich (Steinheim, Germany). 2-Undecanone and isobutyl isobutyrate were obtained from J&K Scientific (Lommel, Belgium), methyl geranate was from Alfa Aesar (Kandel, Germany), 2-methylbutyl isobutyrate was from aromaLAB (Planegg, Germany), (+)-α terpineol was from Merck (Darmstadt, Germany), and propyl 2-methylbutyrate was from TCI (Eschborn, Germany). The purity of all standards was >95%. Ethyl acetate was obtained from Th. Geyer (Berlin, Germany), and LC-grade water was from Roth (Karlsruhe, Germany).

Sample Preparation. A standard mix containing all the above-stated analytical standards (1 mg/L) was prepared in ethyl acetate. Aliquots of this mix were stored at −20 °C and used for the optimization experiments. To validate optimized APGC parameters, a diluted (1:1000 in ethyl acetate) hop essential oil sample of the variety Citra (harvest 2019) was used. To determine the limit of quantification, further dilutions of the standard mix (10 and 50 μg/L) were used.

Instrumentation. An Agilent 7890A GC system (Agilent Technologies, Santa Clara, CA, USA) was coupled to a Xevo G2-XS Q-TOF (Waters Corporation, Manchester, UK) combined with an APGC source. The GC−Q-ToF system was equipped with a PAL Combi-xt System (CTC Analytics, Zwingen, Switzerland) operated by the Maestro 1 Software (V 1.4.56.6) (Gerstel, Mühlheim an der Ruhr, Germany). GC separation was carried out on a DB-5MS analytical column (30 m × 0.25 mm i.d. × 0.25 μm film thickness; Agilent Technologies, Santa Clara, CA, USA).

APGC Default Method. One microliter of the standard mix was injected splitless at an injection temperature of 250 °C. The temperature program was as follows: 40 °C held for 3 min and then raised to 250 °C at a rate of 5 °C/min followed by a final ramp to 300 °C at 70 °C/ min held for 3 min, resulting in approx. 49 min of total run time. Helium (99.999%, Air Liquide, Düsseldorf, Germany) was used as the carrier gas with a constant flow of 1.2 mL/min. The transfer line to the APGC source was maintained at 300 °C with a makeup gas flow of 300 mL/min (nitrogen, 99.999% purity). The Xevo G2-XS QTOF was operated in APGC positive polarity and sensitivity mode, and the ion source temperature was 150 °C. Auxiliary and cone gases (nitrogen, 99.999% purity) were set to 220 and 120 L/h, respectively. The corona current was 2.2 μA, and the sample cone voltage was set to 30 V. For data acquisition, a collision energy of 6 eV, and a mass range from m/z 50 to 650 with a scan time of 0.2 s were used. The column bleed specific mass of m/z 355.0705 was used for internal mass calibration with a scan time of 0.25 s at 10 s intervals.

Data Acquisition with Default Parameters. Since no prior knowledge about the behavior of the analytes regarding APGC ionization was available, default APGC source parameters as suggested by the manufacturer were selected (Table 1). To assess the predominant ionization mechanism for the analytes tested, measurements of the standard mix were carried out under dry (no H_2O in the ion source) and wet (H_2O in the ion source) source conditions, promoting charge transfer and protonation, respectively (Figure 1b). To create wet source conditions, an unsealed GC vial (1.5 mL) filled with LC-grade H_2O was placed in the vial mount of the source enclosure. APGC analysis was performed in duplicates.

Variation of Cone Gas and Cone Voltage. To optimize ionization, four APGC parameters were changed or varied (Table 1). As the auxiliary gas flow and corona current are predominantly responsible for the overall beam stability, settings were determined by checking for the optimal beam stability and intensity of the column bleed in the tune page (data not shown) and subsequently set to 300 L/h and 2 μA, respectively. Cone gas flow settings were varied in a range between 80 and 200 L/h with steps of 20 L/h, while the cone voltage was kept constant at 20 V. After evaluating the optimal cone gas value, cone voltage was varied in a range of 10−30 V with steps of 5 V. APGC analysis was performed in duplicates.

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Optimized APGC Method. After optimization, the auxiliary and cone gases were set to 300 and 100 L/h, respectively. The corona current was set to 2 μA, and the cone voltage was 5 V (Table 1). The optimized APGC method was used to analyze the hop essential oil sample in comparison to the APGC default parameters under dry and wet source conditions and determination of the limit of detection.[38]

Data Analysis. APGC−Q-ToF data evaluation was done with UNIFI (1.9.3.071, UNIFI Scientific Information System, Waters Corporation). To identify standard substances, elemental compositions and structural information (.mol files) of the standard substances were used to calculate theoretical exact masses of the molecular ions and protonated molecules as well as to search for theoretical fragment ions based on in silico fragmentation provided by UNIFI. Spectral data were exported from UNIFI; processing and graphical presentation of the results were done using Excel (Microsoft 2016), python 2.7.16 (pandas 0.24.2, matplotlib 2.2.3, seaborn 0.9.0), jupyter notebook (V 5.7.8), and inkscape (V 0.92.2). EI spectra were extracted from NIST (NIST/EPA/NIH Mass Spectral Library 2017). Values for m/z and intensity were calculated from duplicate data.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c04426.

Chemical formulas, molecular weights, and expected molecular ions, protonated molecules, and fragment ions (Table S1); absolute and relative intensities and M/F% of molecular ions, protonated molecules, and fragment ions (Table S2); estimation of the limit of quantification (Table S3); heatmap of log10 intensities of molecular ions and protonated molecules (Figure S1); EI spectra and spectra recorded under APGC conditions (Figures S2−S4); absolute intensities of molecular ions, protonated molecules, and fragment ions during the optimization of cone gas (Figure S5); and 2D chromatograms of the hop oil sample with default dry and wet and optimized parameters (Figure S6) (PDF)

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Notes

The authors declare no competing financial interest.

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