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Capabilities of direct analysis in real time mass spectrometry and gas chromatography–mass spectrometry in the mint oil test

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Direct analysis in real time (DART) mass spectrometry is compared to gas chromatography–mass spectrometry by the example of mint essential oil analysis.

Direct analysis in real time (DART) mass spectrometry is a recent method, which is promising for the fast analysis of pharmaceuticals,[1,2] flavours and fragrances.[3] DART mass spectrometry does not require any sample preparation procedure. Solid or liquid samples are introduced directly into the DART ion source. Molecular masses and empirical formulas of test compounds (in case of coupling with a high-resolution mass spectrometer) can be determined from the mass spectra.

The number of publications on DART-MS and its comparison with other modern hyphenated techniques is very limited. The available data on its application to quality control of pharmaceuticals are restricted to a very few examples of solid drugs and drug substances analysis and only one example of ointment analysis.[4–7] In our opinion, it is important to investigate the potential of DART-MS in comparison with GC-MS with electron ionization, a well-established method for the identification of mixture components.

Here, we discuss the benefits and limitations of DART-MS used for the analysis of pharmaceuticals, as compared to GC-MS, by the example of mint oil.[†]

According to results (Figure 1, Table 1) of our GC-MS experiments, the mint oil sample contained six major components: limonene, isopulegol, menthone, menthol (two isomers) and menthyl acetate. The determined composition of the major components of the mint oil sample is in good agreement with published data.[8]

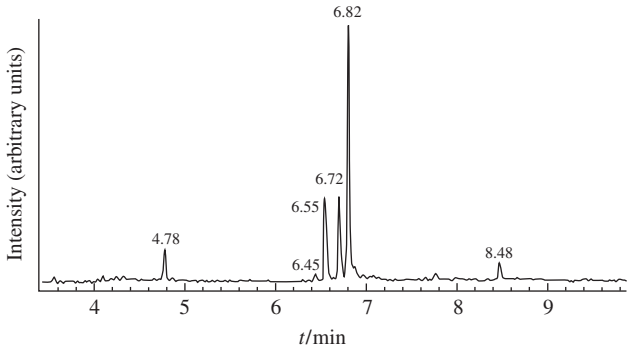


Figure 1 GC-MS total ion chromatogram for diluted mint oil (1:1000). Analysis duration, 10 min.

The use of time-of-flight mass spectrometer in DART-MS experiments made it possible not only to determine the masses of compounds and compare them with the expected molecular masses of the components but also to obtain the mass values of high precision and to calculate the empirical formulas of components using mass drift compensation.

The signals at m/z 137, 139, 153, 155, 172, 174, 226, 228, 305, 307 and 309 were observed in the DART mass spectrum of undiluted mint oil (Figure 2). Surprisingly, there was no $[M + H]^+$ signal corresponding to menthol at *m*/*z* 157; however, it was obvious from the GC-MS experiment that menthol was one of the major components.

We determined the exact molecular masses and suggested possible elemental formulas for intense signals observed in the recorded DART mass spectra. We also attempted to compare the experimental values of *m*/*z* for the most abundant ions (Table 2) with data on the composition of these samples obtained from GC-MS experiments. In order to estimate the elemental

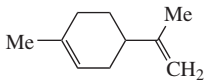
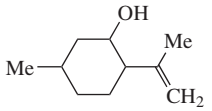
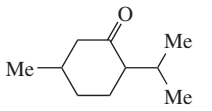
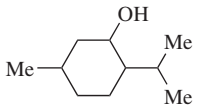
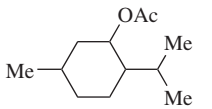
[†] The test sample of mint essential oil (Mentha piperita 100%) was manufactured by 'Aromaty Zhizni' (Moscow, Russia).

For GC-MS and DART-MS experiments, 99.999% purity grade helium was used. Methanol (HPLC grade, Biosolve, Netherlands) was used as a solvent.

Undiluted mint oil was analyzed using a JEOL AccuTOF mass spectrometer equipped with a DART ion source using melting point glass sticks. The flow rate of helium during acquisition was 2 dm^3 min^{-1}. The DART temperature was 250 °C. Spectra were recorded in the *m*/*z* range from 50 to 1000. The duration of sample introduction into the DART source was 3–7 s.

The mint oil sample for GC-MS analysis was diluted (1:1000) with methanol. An Agilent 6890N gas chromatograph equipped with a split/splitless injection port and interfaced with a JEOL GCmate II mass spectrometer was used. The injection volume was 1 μl. Mass spectrometer was operated in the electron ionization mode (70 eV electron energy), and the total ion chromatogram was recorded. Analytes were separated on a 30 m DB5-MS capillary column, 0.25 mm i.d., 0.32 μm film thickness. The split ratio was 1:50. Helium was used as a carrier gas, and the following temperature program was applied: 60 °C (1 min hold), from 60 to 140 °C at 10 K min^{-1} (3 min hold). The injector, transfer line and ion source temperatures were 270, 250 and 250 °C, respectively. Mass spectra were recorded in the *m*/*z* range from 19 to 850 at a scan rate of 1 scan s^{-1}. For the identification of mint oil components, the NIST'98 mass spectral database was used.

Table 1 Major mint oil components identified by GC-MS with NIST library search.

Retention time/min	Compound	Empirical formula	Structural formula	Molecular weight
4.78	Limonene	$C_{10}H_{16}$		136
6.45	Isopulegol	$C_{10}H_{18}O$		154
6.55	Menthone	$C_{10}H_{18}O$		154
6.72, 6.82	Menthol	$C_{10}H_{20}O$		156
8.48	Menthyl acetate	$C_{12}H_{22}O_2$		198

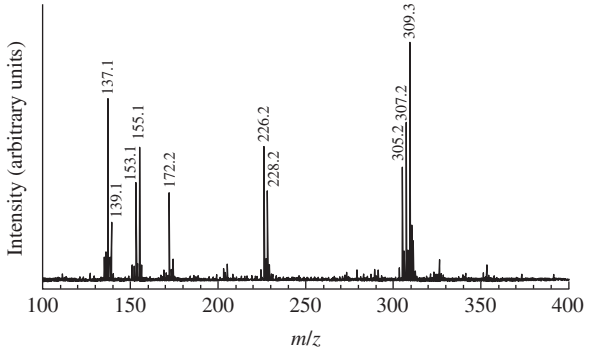


Figure 2 DART-MS spectrum for mint oil. Analysis duration, 5 s.

composition, we applied a tolerance of 30 mmu, which was chosen taking into account the time-of-flight analyzer resolution of 6000 and the averaged value of estimated mass-to-charge ratio.

Table 2 indicates that it was impossible to completely correlate the corresponding compounds with some signals present in the DART mass spectrum, even after comparison with either GC-MS analysis data or any available information on the mint essential oils composition. We could not identify the composition of ions with *m*/*z* 226 and 228 using GC-MS experimental data. There were no respective signals on the GC-MS reconstructed ion chromatograms for *m*/*z* 225 and 227 (possible $M^{+\cdot}$, if ions with *m*/*z* 226 and 228 corresponded to $[M + H]^+$), nor for *m*/*z* 243 and 245 (possible $M^{+\cdot}$, if ions with *m*/*z* 226 and 228 corresponded to $[M - H_2O + H]^+$), nor for *m*/*z* 207 and 209 (possible $M^{+\cdot}$, if ions with *m*/*z* 226 and 228 corresponded to $[M + H_2O + H]^+$). It is likely that more severe temperature conditions and a longer analysis duration are required for the elution of the respective components from the GC column.

Another problem is the nature of ions with *m*/*z* 172 and 174, which were attributed supposedly to menthone (or isopulegol) and menthol cations $[M + NH_4]^+$. The origin and reasons for the production of such ions requires further investigations.

By reference to the experimental data it could be assumed that the compounds with a determined menthol-like fragment generate the ions with a difference of 2 a.m.u. in their *m*/*z* values resulted from dehydrogenation of the corresponding compounds under DART ionization conditions due to the easy formation of coupled double bond system in compounds of this type. However, in the course of analysis of 1% menthol solution in ethanol with DART-MS such groups of ions were not observed. Therefore, the possible dehydrogenation of some classes of compounds in the DART ion source requires further revision.

Table 2 Interpretation of DART mass spectra for mint essential oil samples.

m/z at a resolution of 0.03 a.m.u.	Possible elemental composition	Possible assignment
137.13	$C_{10}H_{17}$	Limonene $[M + H]^+$ Menthol $[M - H_2O - H_2 + H]^+$ Menthyl acetate $[M - AcOH - H_2 + H]^+$
139.15	$C_{10}H_{19}$	Menthol $[M - H_2O + H]^+$ Menthyl acetate $[M - AcOH + H]^+$
153.13	$C_{10}H_{17}O$	Menthone (or isopulegol) $[M - H_2 + H]^+$
155.14	$C_{10}H_{19}O$	Menthone (or isopulegol) $[M + H]^+$ Menthol $[M - H_2 + H]^+$
172.17	$C_{10}H_{20}O_2$	Menthone (or isopulegol) $[M + NH_4]^+$
174.18	$C_{10}H_{22}O_2$	Menthol $[M + NH_4]^+$
226.18	$C_{14}H_{26}O_2$	No candidate from GC-MS experiment
228.20	$C_{14}H_{28}O_2$	No candidate from GC-MS experiment
305.25	$C_{20}H_{33}O_2$	Dehydrogenated menthone dimer $[2(M - H_2) + H]^+$
307.26	$C_{20}H_{35}O_2$	Mixed menthone/(menthone – H_2) dimer $[M + (M - H_2) + H]^+$
309.28	$C_{20}H_{37}O_2$	Menthone (or isopulegol) dimer $[2M + H]^+$

Thus, we can conclude that DART mass spectrometry is a very attractive technique for confirmation of low-molecular-weight compounds. The advantages of DART-MS in comparison with other hybrid methods, including GC-MS, are the following: it does not require sample preparation; the analysis duration is a few seconds, and the molecular formula can be determined using a time-of-flight analyzer. However, the limitations of DART-MS, in particular, as compared to GC-MS, are as follows: (i) sensitivity is lower than that of GC-MS by a few orders of magnitude; (ii) it does not provide any structural information; (iii) it does not differentiate between isomeric and stereoisomeric compounds; (iv) no quantitative information can be obtained when manual sample introduction is used; and (v) DART mass spectra contain not only peaks of $[M + H]^+$ ions; they are much more complex, and a sophisticated interpretation is required, especially, in case of multicomponent samples.

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