Fast Quantification of Whisky Lactone in Oak Wood by Ion Mobility Spectrometer

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Abstract

We have applied the Ion Mobility Spectrometry (IMS) technique to detect the β -methyl- γ -octalactone (Whisky Lactone – WL) vapours in the air and as well WL vapours originating from the oak wood samples. The IMS with Atmospheric Pressure Chemical Ionisation (APCI) based on Corona Discharge (CD) ion source, was operated in the positive polarity. IMS responds to WL with appearance of two peaks in the IMS spectrum, monomer with reduced ion mobility value $K_0=1.39 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ and dimer $K_0=1.09 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$. Using Ion Mobility orthogonal acceleration Time of Flight mass spectrometer (IMS-oaTOF MS) techniques we have identified these peaks as protonated monomer $\text{M}\cdot\text{H}^+(\text{H}_2\text{O})_{0,1}$ and dimer $\text{M}_2\cdot\text{H}^+$ ions respectively. The limit of detection study resulted in LOD for WL of 50 ppb_v. Detection of WL from oak wood samples of different “Quality Level” (QL) categories (categories 1 to 10), indicated strong correlation between the QL category number and the response of the IMS.

1. Introduction

Ageing of the distilled beverages is important process influencing the final quality of the beverages. Many of them (e.g. whiskies and brandies) mature in the oak casks, which contribute to the final taste and aroma of the beverages. Spirits maturation in oak casks and the resulting olfactory perception depends on the content of volatile extractive compounds in the oak^{1,2,3}. The changes in the spirits are due to changes in the composition and concentration of the compounds influencing the taste and aroma. The content of these substances in the oak wood depends on the natural factors such as climate, topography, soil and dendrology⁴. Different oak species differ in the composition of volatile substances, such as the β -methyl- γ -octolactone (whisky lactone or WL), which is an important ingredient in the whisky⁵. In Europe the most abundant oak species are *Quercus petraea* and *Quercus robur*. WL has been found to be more abundant in *Q. petraea* than in *Q. robur*⁶. However, in *Q. petraea* wood exists high natural variability of WL concentration within and between individual trees⁷. For this reason, in the production of oak casks for whisky maturation exists a need for fast, efficient and sensitive technique for WL detection in the oak wood.

There exist numerous studies dealing with detection of WL in beverages like whisky or wine⁸. In these studies, different extractive procedures were applied to extract WL from the liquid phase such as liquid-liquid extraction (LLE), solid-phase extraction (SPE) or solid-phase microextraction (SPME) and stir bar sorptive extraction (SBSE)⁹. In next step usually Gas Chromatography (GC), or GC combined with mass spectrometry (GC-MS) were applied to detect WL^{10,11}. Direct study of the extractive compounds (including whisky lactone) in the wood was reported by Prida et al.¹². They studied WL presence in wood of oaks in north France. For detection of these species they used extractive method based on liquid extraction with dichloro-methane and subsequent detection by GC-MS.

In this paper we present a new, fast, sensitive and simple method of whisky lactone detection based on the Ion Mobility Spectrometry (IMS). IMS is spectrometric method based on the separation of ions in homogenous electric field at atmospheric pressure^{13,14,15,16}. This technique is widely used in the fields of security (detection of explosives, drugs and harmful substances)^{17,18}, however, more and more industrial applications of IMS technique appear. We have applied IMS technique equipped with Atmospheric Pressure Chemical Ionisation (APCI) for direct detection of the whisky lactone from the oak wood. Prior the analysis of oak samples, we have performed laboratory studies with WL standard (Sigma Aldrich) using IMS and IMS combined with oaTOF MS. IMS-oaTOF MS technique allowed us to determine the m/z of the

ions within ion mobility peaks. The laboratory study with the WL standard was followed by IMS study of the oak wood samples of different *Q. petraea* trees. In the IMS spectra of different oak wood samples we have identified the WL peaks and their intensity was proportional to the WL concentration.

2. Experiment

For the detection of whisky lactone we have used Advanced Ion Mobility Spectrometer (AIMS) by MaSa Tech company¹⁹ and IMS combined with orthogonal acceleration time of flight mass spectrometer (IMS-oaTOF MS) developed at Department of Experimental Physics²⁰. Both instruments were equipped with identical ion source based on positive polarity corona discharge (CD), which is used to generate reactant ions (RI) for atmospheric pressure chemical ionisation (APCI). The Bradbury-Nielsen type of shutter grid (SG) was operated at frequency of 80 Hz with a pulse width of 60 μ s. The IMS-oaTOF MS instrument can be operated in three different regimes: i) IMS regime, where only IMS spectra are recorded, ii) TOF regime, where SG of the IMS is fully open and the mass spectra are recorded and iii) in two-dimensional (2D) regime, where 2D IMS-MS spectra are recorded. The operation parameters of the instruments are gathered in the Table 1.

Table 1. Parameters of IMS and IMS-MS used in the experiment.

IMS drift tube length	11.44 cm
Drift field intensity	436 V.cm ⁻¹
IMS operating temperatures	332 K
Drift gas flow rate	600 ml/min
Sample gas flow rate	20 ml/min
CD current	10 μ A
SG pulse width/frequency	60 μ s/80Hz
Average IMS resolving power ($t/\Delta t$)	50
TOF pulse width	10 μ s
TOF pulse frequency	40 kHz
TOF acceleration voltage	2185 V
Mass accuracy	0.1 Da
Average TOF resolving power	800

2.1. Sample introduction

The WL liquid sample was placed in a glass vial and the vapours were sampled from headspace by capillary inlet of the AIMS or IMS-*oa*TOF MS. The sample was kept at room temperature (295 K). The sample gas flow was set to 20 ml/min. According to the literature²¹ the vapour pressure of WL at room temperature is 1.5 ± 0.09 Pa (cis) and 2.0 ± 0.1 Pa (for trans), e.g., 15 and 20 ppm.

In addition to liquid WL we have measured the presence of WL in oak wood samples of different Quality Level categories (1 to 10). The oak samples were separated in 10 different categories based on WL concentration in the wood and was categorised by the supplier. The wood samples were analysed using headspace technique using 1 g of wood as a sample at room temperature. The headspace was sampled by the capillary inlet sniffing for 7 seconds.

2.2. Gases and chemicals

The purified ambient air was used (Zero Air Generator by MaSaTech s.r.o.) as the drift gas at 0.6 l/min gas flow rate and the water content was 0.6 ppm as determined from the ratio of the intensities of $H^+.(H_2O)_4$ to $H^+.(H_2O)_3$ using the IMS-*oa*TOF MS mass spectrum and the method described by Kebarle et al.²² The WL (CAS 39212-23-2) was obtained from Sigma-Aldrich (mixture of cis and trans) at purity $\geq 98\%$.

The studies with the WL standard were complemented by the real wood samples (*Q. petraea*) obtained from an industrial partner. The wood samples were classified in ten Quality Level (QL) categories depending on the concentration of whisky lactone. The first category “Quality Level 1” had concentration in range: 0-7 $\mu\text{g/g}$ while the tenth category “Quality Level 10” had concentration in the range 63-70 $\mu\text{g/g}$. The concentrations range of other “Quality Level” samples were not known, however should be evenly distributed between the level 1 and 10.

3. Results and discussion.

We have recorded the spectra of whisky lactone standard (Sigma-Aldrich) using AIMS and IMS-*oa*TOF MS techniques. The ionisation in these instruments proceeds via atmospheric pressure chemical ionisation (APCI) by reactant ions (RI) formed in the corona discharge of the zero air. The zero air was produced by MaSaTech zero air generator²³, which cleans the ambient air by a system of several traps and finally dehumidified by zeolites to a level of approximately 20 ppm. The positive corona discharge of the zero air results in formation of

$H^+(H_2O)_n$ ($n=3,4$) and $NH_4^+(H_2O)_n$ ($n=1,2$) (Figure 2), with dominant $H^+(H_2O)_{3,4}$ ions. These so-called RI are precursors in the process of chemical ionisation. The APCI ionisation includes the following reactions of the RI with the analyte:

Where M represents a WL. Reaction (1) occurs very efficiently if the proton affinity of the molecule M $PA(M)$ exceeds the proton affinity of the $(H_2O)_{3,4}$ clusters ($PA((H_2O)_3)=9.32$ eV, $PA((H_2O)_4)=9.64$ eV²⁴).

Figure 2 shows the positive polarity IMS spectrum of zero air (black line) and of the whisky lactone. The zero air spectrum exhibits two reactant ion peaks with reduced ion mobilities of $2.00 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ ($NH_4^+(H_2O)_n$) and $1.92 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ $H^+(H_2O)_{3,4}$ and the WL spectrum shows two peaks with reduced ion mobilities of 1.39 and $1.09 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ respectively. These IMS spectra were recorded at the drift gas temperature of 332 K and temperature of the samples was kept at room temperature. These two IMS peaks we can unambiguously link to WL, however, from IMS we were not able to learn the nature of the ions. According to the values of the reduced ion mobilities we have assumed that the first peak is related to WL monomer and the second peak to the dimer.

We have applied IMS-*oa*TOF technique to identify the nature of the WL related IMS peaks. Using this technique, we have measured the 2-dimensional (2D) IMS-MS spectrum of WL (Figure 3). The x-axis represents the time of flight of the ions in the TOF mass spectrometer (related to the m/z of the ions) and the y-axis represents the drift time of the ions in the IMS. On the basis of this 2D spectrum we have assigned ions with particular m/z to the IMS peaks. According to the Figure 3 the IMS peak with ion mobility of $1.39 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ we have assigned to protonated WL ion $M \cdot H^+$ ($m/z=157$) and its hydrated cluster $M \cdot H^+(H_2O)$ ($m/z=175$). Presence of these peaks indicates that the ionisation proceeds according to the reactions (1) and the hydrated ion is formed via reaction:

Where X is a third body (e.g. drift gas constituent N_2 , O_2 ...). The peak with ion mobility of $1.09 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ we have identified as WL dimer ions $M_2 \cdot H^+$ ($m/z=313$). The protonated cluster ions are most probably formed via clustering reaction in the reaction region:

We have carried out limit of detection (LOD) study for WL detection by IMS. The vapour pressure of WL mixture at room temperature (295 K) was 2.0 Pa and their relative concentration 20 ppm. Diluting the sample vapours with the air using micro splitter we were able to prepare concentrations of WL as low as 10 ppb. Measuring the response of the IMS to the WL sample

using the $1.39 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ mobility peak we were able to achieve LOD of 50 ppb for signal exceeding 3 times the average noise level.

After the studies with the WL standard we have started measurements with the real wood samples. These wood samples were analysed by the AIMS using the procedure described in experimental part. In the Figure 4 we see IMS spectra of selected wood samples (Quality levels 2, 4 and 9). These spectra are dominated by the $1.39 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ peaks, which are composed of $\text{M}\cdot\text{H}^+$ and $\text{M}\cdot\text{H}^+\cdot\text{H}_2\text{O}$ ions. The intensities of this peak are proportional to the WL concentration vapours released from wood. If we assume that the release of the WL from wood is in equilibrium (we had constant temperature and pressure), the concentration of the WL vapour above the sample is proportional to the WL concentration in the wood. From the wood samples we have observed additional peaks in the IMS spectrum, however, we have no knowledge about their origin and their study was out of scope of this paper. Most probably they originate from other volatile compounds present in the wood.

The results of the real wood samples are summarised in the Figure 5. The intensities of the IMS WL peaks are given in nA as determined by AIMS. There is clear correlation between the IMS WL peak intensities and the “Quality level” category of the oak wood. These results indicate that the AIMS measurement of the vapours released by the oak wood in the headspace have a potential to be used for fast and efficient identification of the WL content in the wood.

4. Conclusions.

The present work proved that IMS technique can be applied for detection of WL vapours with high sensitivity and short response time. The sensitivity measurements revealed that LOD for WL can be as low as 50 ppb_v. In the positive polarity IMS response to WL with appearance of a peaks with reduce ion mobility of $1.39 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ and at higher concentration second peak with reduced ion mobility $1.09 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$. Using IMS-MS technique we have assign the first IMS peak to protonated monomer ions ($\text{M}\cdot\text{H}^+(\text{H}_2\text{O})_n$, $n=0,1$) and the second to the protonated WL dimer ion $\text{M}_2\cdot\text{H}^+$.

The analysis of real wood samples demonstrated that the IMS is sensitive enough to detect the WL vapours emanating from the wood and thus can be applied directly for detection of WL in the real wood samples. The IMS was able to clearly distinguish between 10 Quality Levels of the wood with different concentration of WL.

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Conflicts of interest

Declarations of interest: none

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