APPNOTE

GERSTEL
MAKING LABS WORK

GERSTEL AppNote Nr. 155

Large Volume Injection with GC/MS Multi Column Switching for Direct Determination of Ethyl Carbamate in Alcoholic Beverages

Thomas Albinus and Kevin MacNamara

Gerstel GmbH & Co. KG, Eberhard-Gerstel-Platz 1, 45473 Mülheim an der Ruhr, Germany

Keywords

Ethyl Carbamate, Distilled Spirits, Capillary GC-MS, LVI, Solvent Vent, Multi Dimensional Column Switching

Abstract

A procedure is presented for quantification of Ethyl Carbamate at low µg/L levels in distilled spirits. A 100 µL large volume injection was used followed by orthogonal 2-dimensional GC-MS with heartcutting. The direct large volume injection ensured sufficient availability of analyte without an initial sample preparation step, and the 2D step allowed clean elution of ethyl carbamate and it's labelled internal standard and compensated for the difficult detection of low mass non-specific ions in a complex matrix. The 2D separation was achieved using the new GERSTEL µFlow- Manager based on metal ferrules for simple connection of the orthogonal columns. The principle and apparatus required for both large volume injection and 2D separation will be described together with results from actual samples.

Introduction

Ethyl carbamate is a naturally occurring carcinogen present in all fermented foods and beverages with it's principal source the reaction between urea and ethanol. In 1985 relatively high levels of ethyl carbamate were reported in certain types of alcoholic beverages sold in Ontario and subsequently led to Canadian Government guidelines of 150 µg/L as maximum allowable level in distilled spirits [1]. For market place acceptance significantly lower levels are desirable. A common procedure within Industry for detection and quantification of ethyl carbamate involves a 1 µL direct splitless injection of the spirit to a polar column followed by mass spectrometric (MS) detection of relevant ions in selected ion mode(SIM). Figure 1 shows the mass spectrum of ethyl carbamate and it is clear that this simple molecule shows relatively non-specific ions and unfavourable abundances. M/Z 62, 74 and 89 are possible candidates but 74 is a common ion susceptible to overlapping interferences and 89 with very low abundance also bleeds naturally from the required polar chromatographic phase [2]. Therefore, while direct splitless injection is very cost effective for multiple sample screening, in reality only the single m/z 62 ion can be comfortably monitored, and requires a long run time to ensure a clean elution window. Single ion analysis may suffice for internal QC but is unacceptable in the context of a regulatory environment. The concept of three ions within expectable ratios for confirmation of positives has been clarified [3]. The official procedure of the Canadian Government Laboratory uses multiple ion monitoring and expected ion ratio confirmation after SPE cleanup and analyte concentration.

GERSTEL AppNote Nr. 155

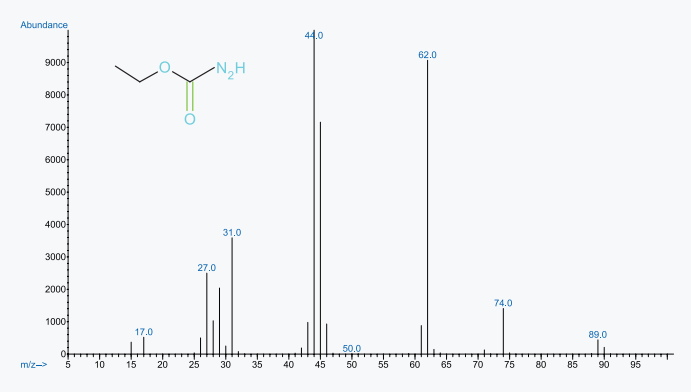

Figure 1: Mass spectrum of ethyl carbamate.

In this publication a useful combination of sample introduction and separation techniques is described which allows low µg/L detection of ethyl carbamate without any sample preparation and with three ion confirmation of positives. The large volume injection (LVI) involves slow speed programmed sample introduction with programmed temperature vaporization and concurrent venting of the ethanol-water matrix with concentration of the analytes in the injection port liner [4, 5]. The 2D separation of ethyl carbamate was previously successfully applied [6], but here the new GERSTEL µFlow-Manager is used for the heart cutting transfer. This significantly simplifies the connection of the two columns while providing very low air background connections and has evolved 2D separations into a routine procedure.

Experimental

Standards and Whiskey samples. Ethylcarbamate (EC) standards were prepared at 10 and 1 mg/L and 100 µg/L in a 60:40 vol % water/ethanol matrix. A whiskey sample containing a relatively low level of EC (16 µg/L) was used as a real sample.

Instrumentation

Analysis was performed on a double-oven GC-MS consisting of two 6890 GCs and a 5975B mass spectrometer (both Agilent Technologies). The two GCs were connected by a Cryo Trap System with LN_2 cooling (CTS1, GERSTEL). A GERSTEL CIS4 PTV-type inlet with Universal Peltier Cooling (UPC) was used as injection port and injections were performed by a MultiPurpose Sampler (MPS, GERSTEL). Heartcutting was performed using a MultiColumnSwitching pneumatics (MCS, GERSTEL) and the novel µFlow-Manager µMulti-Column Switch (µMCS) fraction distribution device (Figure 2, GERSTEL).

Figure 2: GERSTEL µMCS fraction distribution device.

Analysis conditions

CIS 4	solvent venting, 500 mL/min 40 °C (4 min); 12 °C/sec to 280 °C (5 min)
Pneumatics	constant pressure, 166 kPa
1st Column	30 m Rxi-5ms (Restek) d_i = 0.25 mm d_f = 1.0 µm 60 °C (4 min); 5 °C/min to 120 °C; 30 °C/min to 280 °C (5 min)
CTS 1	250 °C (10.5 min); 10 °C/s to -75 °C (2 min); 10 °C/s to 250 °C (3 min)
2nd Column:	30 m Stabilwax (Restek) d_i = 0.25mm d_f = 0.25 µm 60 °C (1 min); 5 °C/min to 150 °C; 30 °C/min to 240 °C (1 min)
Cut 1	11.2 - 11.9 min (60.6 kPa)

To define the time window for heartcutting, an ethyl carbamate standard of 100 mg/L in artificial matrix (60 % water, 40 % Ethanol) was prepared and a 1 µL aliquot was injected into the PTV in splitless mode. The retention time for ethyl carbamate was recorded and used to determine the heartcutting time window from 11.2-11.9 min.

During the runtime except for the heartcutting window, the MCS2 pneumatics box applies a software-adjustable helium counterflow through the CUT connector of the µMCS. The counterflow prevents eluting compounds from the 1st dimension column from entering the 2nd dimension column. Only clean carrier gas will enter the 2nd column. In order to maintain the correct headpressure for

the 2nd dimension column, the MCS pneumatics box incorporates a pressure regulator, which is connected to the VENT outlet of the µMCS

During the GC heartcutting period (11.2-11.9min), the helium counterflow and the MCS pressure control are switched off. The 1st dimension eluate is transferred quantitatively to the 2nd dimension. In contrast to a Deans-switch, no additional flow is added to the 1st dimension column i.e. small internal diameter columns can be used while still maintaining good velocity in the 2D-separation and also perfect vacuum conditions in the MS detector.

The µMCS connector is a novel device based on the well known GERSTEL MCS. It involves metal-ferrules that can be mounted by simply pushing them onto the column without the need to adjust column insertion length.

Between the two GC ovens, a CryoTrap System (CTS, GERSTEL) kept at -150 °C was used to focus the heartcut compounds enabling the transfer of analytes to the 2nd dimension column in a narrow band. The 2nd dimension GC oven and the MSD are automatically started when the CTS starts heating.

Results and Discussion

LVI Optimization

LVI using a PTV inlet system in solvent vent mode can be used to increase the injected sample volume and is applicable to a wide variety of solvents. A basic equation has been developed [7] relating the maximum injection speed for a specific solvent with it's physical properties, the PTV initial temperature and the split vent flow. When this speed is correctly optimized the solvent will be selectively removed and analytes of interest concentrated in the liner for transfer to the column and detection system. This equation is used in the GERSTEL MAESTRO software shown in Figure 3 for rapid method development by calculating the injection speed from the above parameters (see GERSTEL AppNote 11/2012 for discussion of PTV temperature and pneumatic parameter interplay in solvent vent operation). A too low injection speed may lead to loss of low boiling analytes, while a too high injection speed leads to general loss of analytes as they will exit the split line with unvaporized solvent. In general the more apolar solvents follow the equation with the calculator giving injection speeds compatible with good analyte recoveries. A safety margin correction is available in the software and this is usually set to 0.7 – 0.8.

Calculator prediction for water and samples containing large amounts of water such as distilled spirits are not successful, even with the recommended factor. The reason for this is that water has an abnormally high latent heat of vaporization which causes an additional cooling effect in the liner during it's removal. A second reason is related to the maximum saturation value of water in the helium venting gas, which results in the need for very high split vent flows. Not taking these considerations into account has led many practitioners to conclude that water cannot be successfully vented. Using a vent flow of 500 mLs/min, a PTV temperature of 40 °C and a correction factor of 0.8, the calculator predicts an injection speed of 21 µL/min for water. However experimental optimization gave best results when the injection speed was lowered to 12 µL/min for the same conditions and factor. A correction factor of 0.43 would have been necessary to predict this correct injection speed and in comparison with the 0.8 factor and a doubling of the injection speed, more than 80 % more analytes were recovered.

GERSTEL AppNote Nr. 155

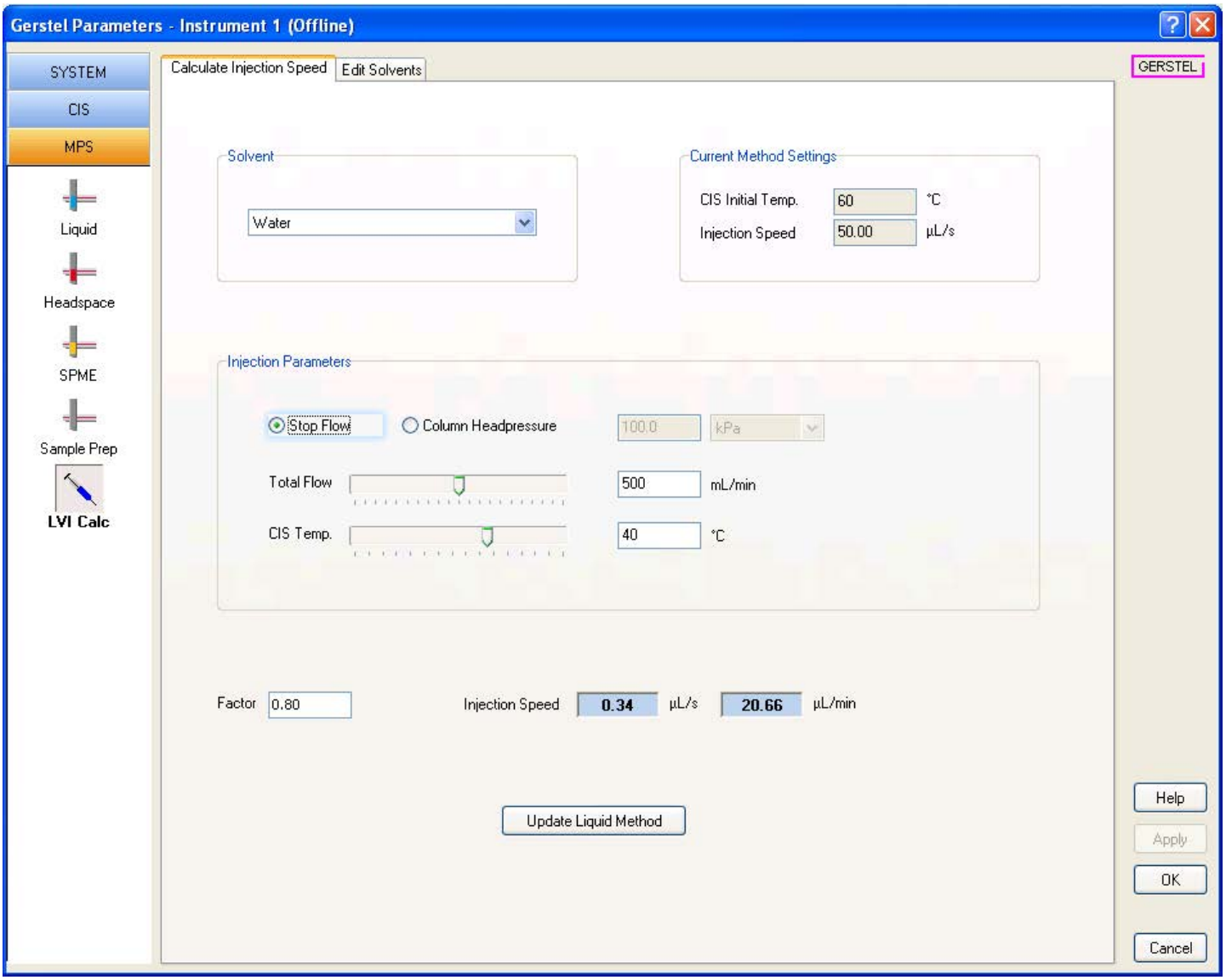

Figure 3: Solvent vent calculator in GERSTEL MAESTRO software.

Two-Dimensional GC-MS

Figure 4 (top) shows the first column monitor FID trace in overlay form for 100 µL injections of 10 ppm, 1 ppm and 100 ppb ethyl carbamate standards in 40 % - 60 % methanol-water. As previously described these traces represent a split of about 1:10 of the first column eluant and even under these conditions the small peak for the 100 ppb level is discernible. Figure 4 (bottom) is the corresponding MS full scan trace after transfer of the 100 ppb injection to the second column. Even in scan mode sensitivity is more than adequate and a clean mass spectrum is obtained.

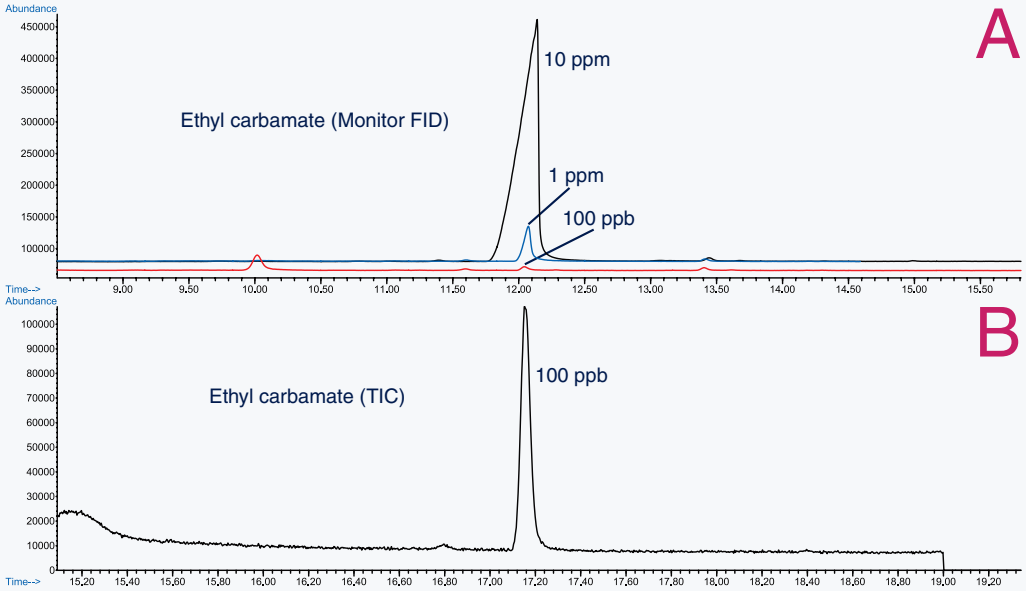

Figure 4: First column monitor FID traces in overlay (A), corresponding MS second column full scan trace (B).

Figure 5 shows a similar TIC of ethyl carbamate after heartcutting to the second column displaying simultaneous SIM and Scan traces.

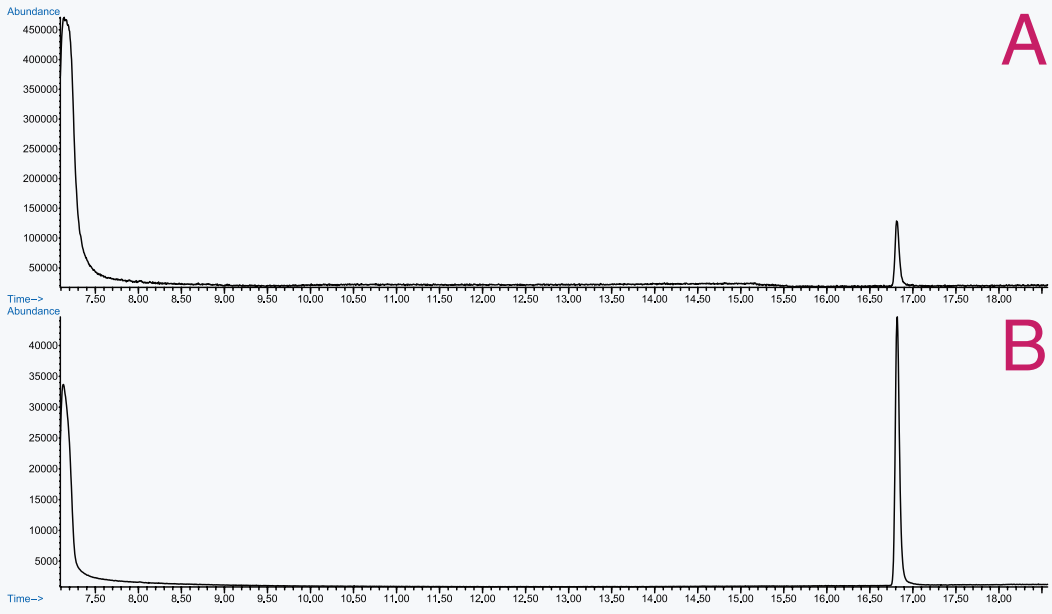

Figure 5: Simultaneous scan (A) and SIM (B) traces for the previous 100 µg/L large volume injection after heart cutting to the second column.

GERSTEL AppNote Nr. 155

Figure 6 shows both the first column monitor trace and main column TIC after 100 µL injection of a whiskey with added internal standard. The complexity of the monitor trace is evident but the 2D gives a clean elution window for the analytes. The identity of the EC was additionally confirmed by connecting a nitrogen-specific (NCD) detector instead of the MSD to the outlet of the 2D column (Figure 7).

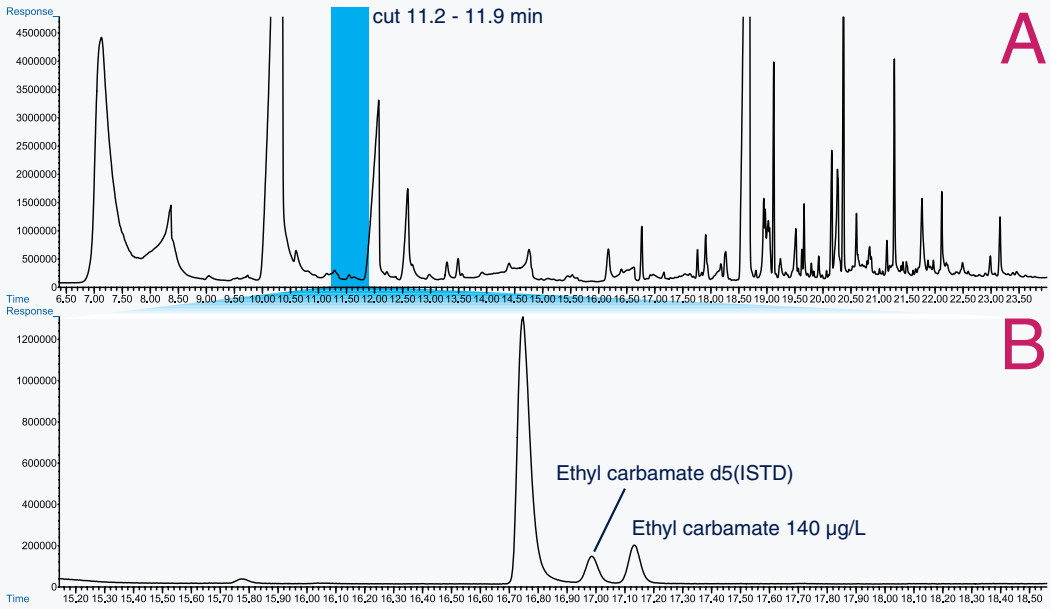

Figure 6: Whiskey with ca. 140 µg/L EC-, and ISTD EC d5monitor trace, column one (A) and MSD scan trace of the heart cut section on column two (B).

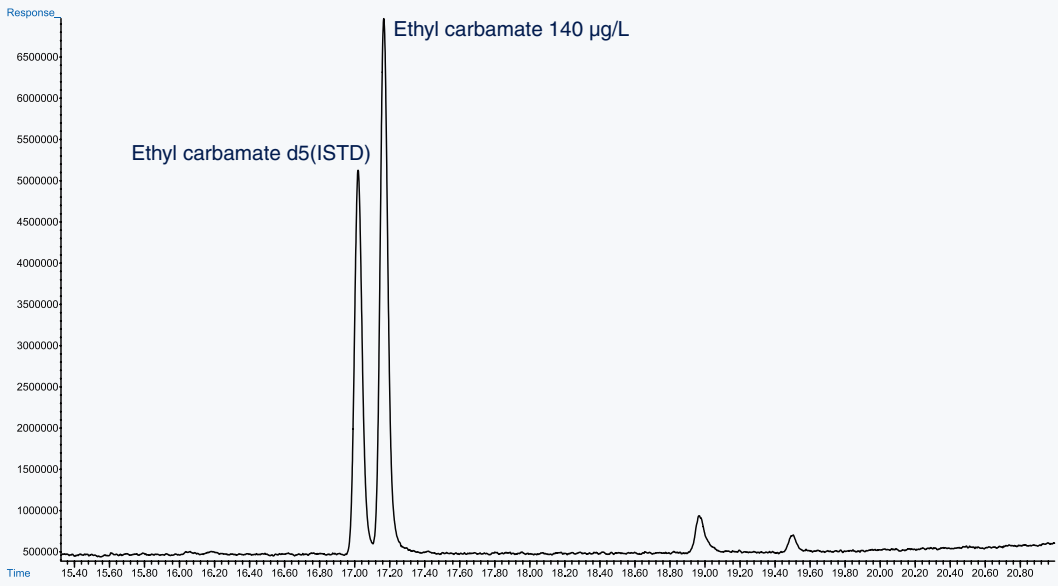

Figure 7: NCD trace corresponding to figure 6B.

Figure 8 (top) shows the 1D FID monitor signal after a 100 µL injection of a blended whiskey with 16 µg/L ethyl carbamate, the chromatogram on the bottom is the related SIM trace for ethyl carbamate.

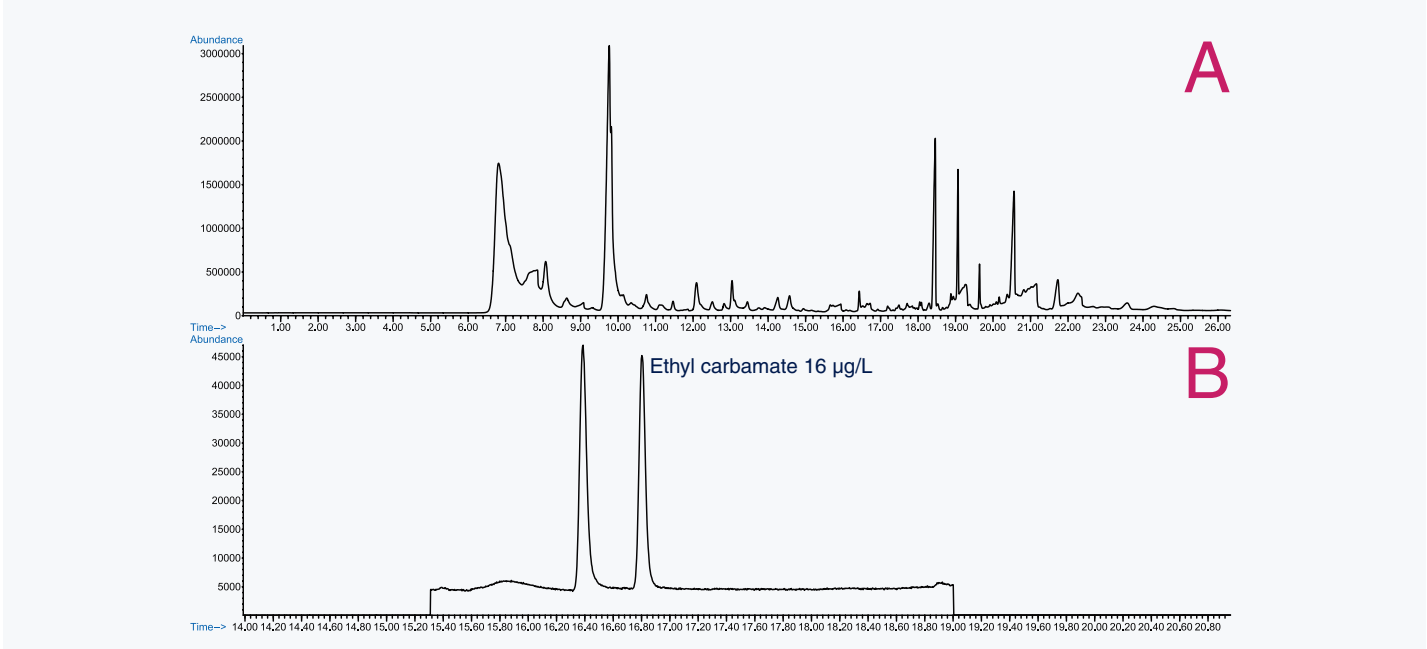

Figure 8: 1D Monitor-FID (A) and 2D-SIM chromatogram Whiskey sample (B) @ 16µg/L .

The new sealing technique used in the µMCS using metal-ferules did not contribute any signs of leakage or of air ingress during the MSD tune compared to single-column operation, no matter if the tune was performed in 'vent on' or 'vent off'-mode, which is a significant difference to former 2D-systems based on graphite-ferrules.

Conclusions

Determination of EC in distilled spirits is usually rather difficult due to matrix complexity and non-specific target ions for MS detection. Applying the two techniques of large volume injection of water and a 2D-separation within one method removes the need for sample preparation and adds selectivity to exceed the required sensitivity for legal requirements.

References

[1] H.B.S Conacher, B.D. Page, Proceedings of Euro Food. Tox. Zürich, 237 (**1986**)

[2] B.P. Lau et al. J. Chromatogr. 402, 233 (**1987**)

[3] D. Hoyt et al, JAMA, Vol 258, No 4 (**1987**) 504.

[4] H.G. Mol et al, Trends in Analytical Chemistry, Vol 15, No 4 (**1996**)

[5] W. Engewald et al, J. Chromatogr. A, 842 (**1999**)

[6] K. MacNamara, A. Hoffmann, Proceedings of the Riva del Garda Capillary Conference (**1991**)

[7] J. Staniewski, J.A. Rijks, J. Chromatogr. 623, 105 (**1992**)