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Advances in headspace sampling for enhanced residual solvent analysis in food packaging

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ABSTRACT

Purpose: This work is focused to highlight performance and benefits of a new valve-and-loop static headspace sampler coupled to a GC-MS/FID dual-detector configuration, for the determination of residual solvents in food packaging according to the regulatory requirements (EN 13628-1:2002)[1].

Methods: The dual detector GC-MS/FID allows for simultaneous identification and confirmation of known and unknown impurities, increasing the confidence in compound identification and solving possible analytes co-elution. Reliable quantitation is achieved through automated Multiple Headspace Extraction (MHE) calibration and reporting is easily automated through the chromatography data system software, for a fully automated workflow.

Results: MHE calibration showed excellent linearity with correlation coefficient $R^2 \geq 0.995$ for all analytes in both solvent standard and samples, exceeding the minimum required value. Traces of residual solvents were found in three of the six analyzed food packaging samples, in the range 0.76 – 29 mg/m^2.

INTRODUCTION

Packaging materials and food containers are essential to ensure safety, quality and product shelf-life. Volatile organic compounds (VOC) used in printing inks, varnishes, dyes and adhesive applied to the final package can leach from the surface and contaminate the food product during manufacturing, shipping and storage determining significant health risks and negatively impacting on the taste, aroma and appearance of the product[2].
Besides the good manufacturing practices, United States and the European Union have implemented regulations to address the use and to quantitate residual solvents in packaging material.
Residual VOCs in food packaging are traditionally analyzed by headspace gas-chromatography (HS-GC), representing a fast and simple technique without the need for time-consuming sample preparation. Innovative design features now available in modern valve and loop headspace autosamplers provide high analytical performance when it comes to routine solvent analysis.

MATERIALS AND METHODS

A Thermo Scientific™ TriPlus™ 500 Headspace (HS) autosampler was coupled to a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph. A Thermo Scientific™ Dual Detector Microfluidics device (P/N 19071030) was used to split 1:1 the carrier gas flow from the analytical column between a Thermo Scientific™ Instant Connect Flame Ionization Detector (FID) and a Thermo Scientific™ ISQ™ 7000 Single Quadrupole GC-MS system, as shown in **Figure 1**. Chromatographic separation was achieved on a Thermo Scientific™ TraceGOLD™ TG-1MS GC capillary column, 30 m × 0.32 mm × 3.0 µm (P/N 26099-4840).
Additional HS-GC-MS/FID conditions are given in **Table 1**.

Sample Preparation

Two standard mixtures, each containing different residual solvents that can be found in packaging materials (mixture 1 and mixture 2 at 7.14% v/v and 9.09% v/v, respectively), were purchased from Sigma Aldrich® (P/N 48994-U and 48995-U). A volume (1 µL) of each standard solution (corresponding to 71.4 µg and 90.9 µg of mixture 1 and 2, respectively) was spiked into the same 10 mL empty sealed headspace glass vial and used as retention time reference for compound identification as well as for MHE linearity assessment with total vaporization. A complete list of analyzed compounds is reported in **Table 2.**

Samples of packaged foods (pizza, cookies, bread, salad, and salami) were purchased locally and the packaging (cling film, wraps, and trays) was separated from the food and analyzed following the EN 13628-1:2002 method. A sample surface of 40 cm^2 (2 × 20 cm) was cut, coiled, and sealed into a 10 mL crimp cap headspace vial (vials P/N 10CV, caps P/N 20-MCBC-ST3). As specified in the EN 13628-1:2002 method, the ratio between the sample area (in cm2) and the vial volume (in mL) was maintained between 3 and 5.

Data Analysis

The data was acquired, processed, and reported using the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2.

Table 1. Instrument parameters for the HS-GC-MS/FID configuration.

TRACE 1310 GC	
Carrier Gas, Carrier Mode	He, Constant Pressure at 110 kPa
Split Ratio	20:1
Septum Purge Mode	Constant at 5 mL/min
Oven Temperature Program	50° C (1 min), 30° C/min, 110° C, 20° C/min, 250° C
FID	
Temperature	250° C
Air Flow	350 mL/min
H_2 Flow	35 mL/min
N_2 Flow	40 mL/min
Acquisition rate	25 Hz
ISQ 7000 Single Quadrupole MS	
Ion Source	ExtractaBrite
Ion Source Temperature	250° C
Ionization Mode	EI at 70eV
Transfer Line Temperature	250° C
Acquisition Mode	Full scan (m/z 25-350)

TriPlus 500 Headspace Autosampler	
Incubation Temperature	120 ° C
Incubation Time	40 min
Vial Shaking	Medium
Vial Pressurization Mode	Pressure
Vial Pressure (Aux Gas Nitrogen)	55 kPa
Vial Pressure Equilibration Time	1 min
Sampling Loop Volume	1 mL
Loop/Sample Path Temperature	120° C
Loop Filling Pressure	34 kPa
Loop Equilibration Time	1 min
Extraction Cycles	4 for MHE
Purge Flow Level	4
Injection Mode	Standard or MHE (for calibration)
Injection Time	1 min

RESULTS

MHE Linearity Assessment according to EN 13628-1:2002 method

A reference solvent standard mix was analyzed using the total vaporization technique, applying the MHE conditions reported in Table 1. MHE allows the extrapolation of the total content of analytes in a liquid or solid matrix through multiple headspace cycles. At each extraction, the area counts of target analytes decrease exponentially, allowing for a linear extrapolation of a total area count on a semilogarithmic plot **(Figure 2)**. The amount of analyte present in the sample is calculated by direct comparison of the total peak area responses to external standards previously analyzed in a similar way but without matrix.

MHE linearity was assessed by plotting the natural logarithm of the peak areas for the standard solution versus the number of headspace cycles (n = 4). Chromeleon CDS interactive charts and reprocessing features allowed for fast MHE calibration plots and correlation coefficient calculations without the need of external calculation tools, as shown in **Figure 3**.

For all the investigated compounds, the calculated correlation coefficients (R^2) were 1.000 for FID data and ≥0.997 for EI full-scan MS traces (**Table 2**). In both cases calculated correlation coefficients met the method requirement ($R^2 \geq 0.98$) confirming an excellent linearity.

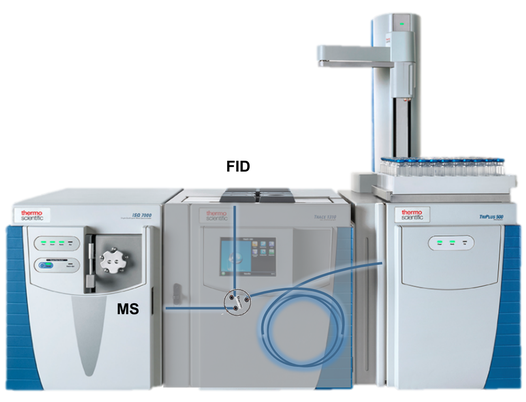

Figure 1. Dual detector HS-GC-MS/FID configuration.

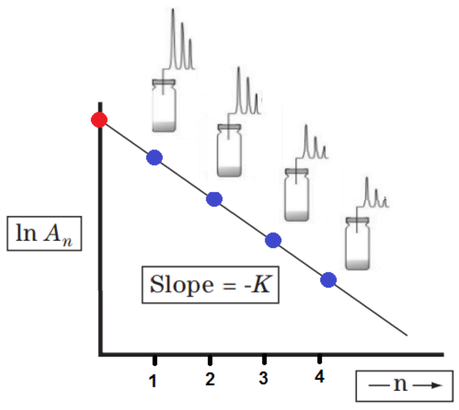

Figure 2. Principle of the MHE extraction.

Calculation of the total peak area e_n for one residual solvent in the standard mix

(equation 1) $$e_n = \frac{(e_1)^2}{(e_1 - e_2)}$$

e1= peak area for the residual solvent in n=1 extraction cycle

e2= peak area for the residual solvent in n=2 extraction cycle

Calculation of the total peak area a_n for one residual solvent in the sample

(equation 2) $$a_n = \frac{(a_1)^2}{(a_1 - a_2)}$$

a1= peak area for the residual solvent in n=1 extraction cycle

a2= peak area for the residual solvent in n=2 extraction cycle

Amount Q of residual solvent in the packaging material (in mg/m^2)

(equation 3) $$Q = \frac{a_n * p}{e_n * S}$$

p= mass of the solvent in the standard mix (in mg)

S= area of the specimen (in m^2)

GC oven ramp was optimized to improve sample throughput (total run time <7 min) but ensuring an adequate chromatographic resolution for all peaks (Rs>1). An incubation time of 40 minutes per MHE step was optimized to cover the majority of food packaging material types.

Table 2. List of solvents included in the method and MHE correlation coefficients using Full Scan MS acquisition.

#	Compound	RT (min)	Correlation Coeff. (R^2)
1	Methanol	1.76	0.997
2	Ethanol	2.15	0.997
3	Acetone	2.41	0.998
4	2-Propanol	2.45	0.999
5	Methyl acetate	2.77	0.999
6	1-Propanol	3.02	0.998
7	2-Butanone	3.36	0.999
8	2-Butanol	3.45	1.000

#	Compound	RT (min)	Correlation Coeff. (R^2)
9	Ethyl acetate	3.52	0.999
10	2-Methyl-1-propanol	3.68	0.999
11	2-Methoxyethanol	3.75	0.997
12	Tetrahydrofuran	3.83	0.999
13	Isopropyl acetate	4.04	0.998
14	1-Methoxy-2-propanol	4.24	0.997
15	Cyclohexane	4.34	0.998
16	Propylacetate	4.60	0.999

#	Compound	RT (min)	Correlation Coeff. (R^2)
17	4-Methyl-2-pentanone	4.93	0.998
18	Isobutyl acetate	5.26	0.999
19	Toluene	5.42	0.997
20	Butyl acetate	5.74	0.999
21	2-Methoxyethyl acetate	5.75	0.997
22	2-Ethoxyethyl acetate	6.47	0.998
23	Cyclohexanone	6.69	0.999

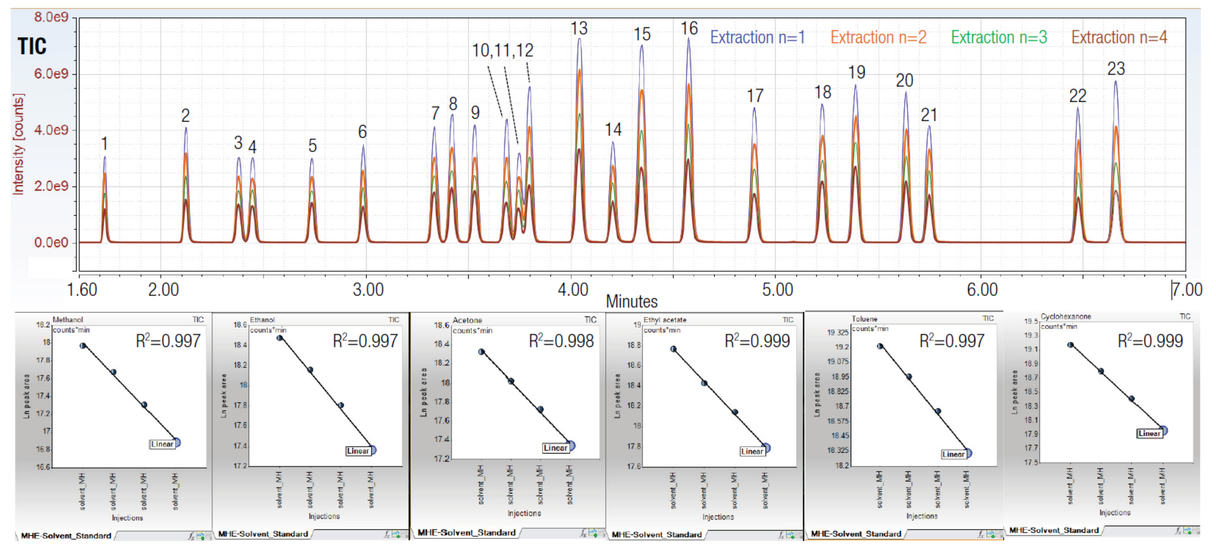

Figure 3. Full scan TIC chromatogram of reference standard and corresponding MHE calibration curves for selected compounds.

Quantification of residual solvent in food packaging materials using MHE

The packaging materials were prepared as described and analyzed using the MHE conditions reported in Table 1. The microfluidic device allowed for splitting the gas flow 1:1 to the FID and the ISQ single quadrupole mass spectrometer, ensuring a minimal effect on the retention times (max RT shifts 0.04 min) by choosing either the FID or MS chromatogram as reference.

The sample and the standard FID chromatograms were compared to verify the presence of known residual solvents. No residual solvents were found in the majority of samples, some traces of ethyl acetate were found in the sliced salami wrap (lid and tray), ethanol and acetone were present in salad wrap **(Figure 4).** MHE linearity in these samples was assessed as previously described. Correlation coefficient (R^2) resulted 0.997 and 1.000 for sliced salami (lid and tray respectively), 0.997 for ethanol and acetone in salad wrap.
The concentration (in mg/m^2) of residual solvents detected in the samples was calculated applying the equations 1, 2, and 3 as reported in the EN method. Ethyl acetate in the sliced salami wrap resulted to be 0.76 mg/m^2 (lid) and 29 mg/m^2 (tray). In salad wrap, ethanol and acetone resulted to be 0.97 mg/m^2 and 1.9 mg/m^2 respectively. All levels were well within the safety limits reported for residual solvent and non-volatile food additives[3].

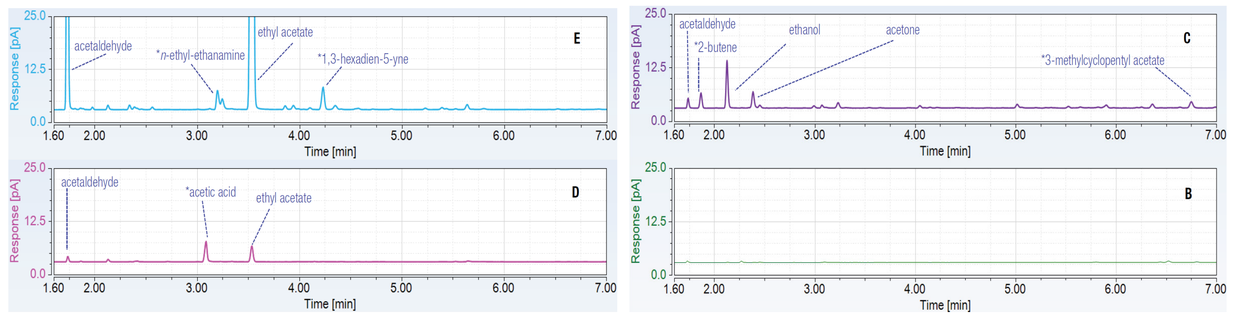

Figure 4. FID chromatograms: empty blank vial (B), salad wrap (C), sliced salami wrap: lid (D) and tray (E).

Full-scan data were used to putatively confirm the identity of detected solvent impurities, increasing the confidence in compound identification. When searching the mass spectrum of the peak eluting at RT = 1.72 min against NIST17 library, the best library match was acetaldehyde (not included in the standard mixtures) with a SI score of 953 (sliced salami tray E) and 729 (sliced salami lid D). Using the same approach, ethanol and acetone in salad wrap (C) and ethyl acetate in sliced salami (lid D and tray E) were also putatively confirmed with a SI score of 929, 913, 874, and 950, respectively.

These chemicals are actually released by the packaging since they are typically used in solvent-based inks imprinted on the external layer of flexible packages. Additional unknown compounds (*) detected in the samples were confirmed using spectral library comparison against NIST17 library. Peaks not annotated were below the integration threshold of 0.04 pA * min.

CONCLUSIONS

The results obtained with the TriPlus 500 HS autosampler are compliant with the EN 13628-1:2002 standard method requirements.

- MHE allows for absolute quantitative analysis of residual solvent impurities in solid samples, overcoming the matrix effect and eliminating the need of sample preparation. Excellent linearity with correlation coefficient $R^2 \geq 0.995$ was obtained for all analytes in both solvent standard and samples, meeting the minimum required value of $R^2 \geq 0.98$.
- The dual detector GC configuration MS/FID increases the confidence in compound identification, allowing for the detection of possible analyte co-elution, otherwise difficult to assess in the absence of MS data. Moreover unknown peaks in the samples have been putatively confirmed (using spectral library match score thresholds of >950 SI) through comparison with NIST17 spectral library.
- The low bleed and superior inertness of the TraceGOLD column allowed for highly reliable results. The high analytical column efficiency allowed for fast GC oven ramp with adequate chromatographic separation (Rs ≥ 1.0) for all the analyzed compounds, reducing analysis time to less than 7 min.
- The automated cycle time optimization allows for continuous sample processing ensuring the overlapping between the MHE cycles of the same sample. The overlapping capability is maintained between the final injection of one sample and the incubation of the next one increasing the sample throughput.
- Chromeleon CDS software ensures data integrity, traceability, and effective data management from instrument control to the final report. The integrated charts and the advanced report capability allowed for easy and integrated MHE data reprocessing, thus eliminating the need for external calculation tools.

REFERENCES

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2. Food Contact Materials - Regulation (EC) No 1935/2004 – European Implementation Assessment Study, May 2016
3. Title 21, Code of Federal Regulation, Direct Additive Part, 170.3, Indirect Additive, Part 174-179
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TRADEMARKS/LICENSING

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