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Centri Application
Food analysis by extraction & enrichment
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Application Note 271

Quantifying trace volatile aromatics (BTEX-styrene) in foodstuffs caused by migration from packaging using extraction and enrichment techniques to enhance GC–MS analysis

This study demonstrates the Centri® sample extraction and enrichment platform's unique multi-step enrichment feature to enhance the discovery of trace-level, packaging-derived contaminants in food and beverage samples by gas chromatography–mass spectrometry (GC–MS). Common volatile aromatic impurities (benzene, toluene, ethylbenzene, xylene and styrene) were analysed in tomato paste, orange juice and coffee using headspace–trap mode, with and without multi-step enrichment. Limits of detection were found to be in the order of 0.03 (BTEX) to 0.4 ppb (styrene) from a single vial; however, using multi-step enrichment, the detection limits were determined to be four times lower.

Introduction

Packaging is ubiquitous in the food and beverage industry, being indispensable for the storage, transportation and handling of products. However, packaging can be a source of volatile organic compounds (VOCs) that have the potential to migrate into the foodstuff through direct or indirect contact, and can affect the flavour and aroma of products and also pose health risks to consumers. VOCs emitted from packaging materials are regulated in the EU (under EC 1935/2004), in the US (under 21CFR175) and in China (under GB 9685-2016).

BTEX compounds (benzene, toluene, ethylbenzene, xylene isomers) and styrene are potentially harmful compounds that may remain in packaging as manufacturing residue or result from degradation of the packaging material post-manufacture. GC–MS is routinely used in the detection of these compounds as well as other potential food contaminants. Analytes can be extracted directly from food packaging,[1] but it is those that have transferred from packaging to foodstuffs that are directly relevant to consumers, and detection of these trace compounds requires different approaches.

The Centri sample extraction and enrichment platform delivers fully automated headspace extraction for GC–MS analysis and is ideal for the determination of trace-level packaging-derived compounds. With Centri, headspace extraction benefits from the option of incorporating a cryogen-free focusing trap into the sample flow path prior to GC(–MS) injection. Through the inclusion of hydrophobic sorbents, water can be selectively eliminated while analytes of interest are retained. Upon injection to the GC, the trap is rapidly heated and the carrier flow reversed ('backflushed') to ensure analytes are delivered to the GC column in a concentrated, narrow band. Together, these features improve chromatography over traditional headspace methods, as well as allowing the use of large headspace extraction volumes (from >1 mL up to 5 mL in a single extraction and multiples thereof) and low-split or splitless injection, enhancing sensitivity without affecting chromatographic performance.

A novel feature of Centri is the multi-step enrichment (MSE) capability. This allows analytes from multiple replicate samples to be sequentially extracted and concentrated (enriched) on the focusing trap prior to desorption and GC–MS analysis. The ability to purge water from the trap to vent prior to injection ensures that chromatographic performance is not compromised, delivering maximum sensitivity enhancement for a more confident identification of trace-level compounds.

In this study, we demonstrate the fully automated sampling and detection of BTEX-styrene compounds in foodstuffs (tomato puree, orange juice and instant coffee) by headspace–trap (HS–trap) and HS–trap with MSE (using multiple vials), and the performance of these methods is compared. We observe robust improvements in sensitivity (peak areas, signal-to-noise ratios) with enrichment and detect trace-level BTEX-styrene more often with this approach than with a single vial extraction. The increase in peak areas allowed for more accurate quantitation of the target components present at trace-levels.

Experimental

Samples:

Calibration standards (Results sections 1–4):

A stock solution of benzene, toluene, ethylbenzene, *m*-xylene, *p*-xylene, *o*-xylene (collectively, BTEX) and styrene, each at 1 ppm, was prepared in HPLC-grade methanol. The volatility of compounds ranged from b.p. 80.1°C (benzene) to b.p. 145°C (styrene). Calibration standards were prepared by volumetric dilution of this stock solution in HPLC-grade water to produce six levels from 0.2 to 10 ppb.

Reproducibility and linearity of response were assessed both with a single HS–trap extraction (1 x 5 mL from one vial) and with multi-step enrichment (MSE) (1 x 5 mL from each of three identically prepared vials, giving a total volume of 15 mL enriched on the trap prior to GC injection). Reproducibility was determined using six replicates of a standard prepared to a concentration of 10 ppb. For each standard, an 8 mL liquid volume was dispensed into a 20 mL headspace vial and crimp-capped.

Food and beverage samples (Results section 5):

The following food and beverage samples were prepared as described below, again with 8 mL sample used per vial.

- Tomato paste (originally packaged in a squeezable metal tube) was added to 15% w/v NaCl in HPLC-grade water (3:4 ratio, paste:NaCl solution, by weight). Each vial thus contained approximately 3.4 g tomato paste.
- Orange juice from concentrate (originally packaged in a coated cardboard carton) was mixed with NaCl powder (0.2:1 ratio, NaCl:juice, by weight).
- Coffee – instant coffee was prepared by dissolving 1.8 g coffee granules in 200 mL near-boiling tap water. Preparation was carried out directly in disposable coated cardboard cups, topped after stirring with disposable polystyrene lids, and left for 55 minutes.

Extraction and enrichment:

Instrument:	Centri (Markes International Ltd.)

Headspace–trap:

Sample:	8 mL
Incubation:	60°C for 30 min, with agitation at 300 rpm
Extraction volume:	5 mL
Enrichment:	3 x 5 mL (total volume 15 mL), sequential extraction from three identically-prepared vials
Injector:	240°C (2 min)

Preconcentration:

Flow path:	150°C
Focusing trap:	Material Emissions (part no. U-T12ME-2S)
Purge flow:	50 mL/min (2 min)
Trap low:	30°C
Trap high:	300°C (3 min)
Split flow	18 mL/min (~5:1)

GC:

Column type:	MEGA-5® MS – 30m x 0.25 mm x 0.25 µm
Column flow:	2.5 mL/min
Oven programme:	35°C (3 min), 10°C/min to 100°C, 30°C/min to 220°C (15 min)

Quadrupole MS:

Transfer line:	280°C
Ion source:	250°C
Mass range:	amu 45–350 (2 mins), then amu 35–300

Background to Centri®

Markes' versatile Centri automation platform combines extraction, enrichment and injection for a wide range of complex GC–MS applications including solid, liquid and gaseous samples.

Centri uses leading GC robotics to maximise instrument usage and throughput, with automated extraction options including HiSorb™ high-capacity sorptive extraction (immersive or headspace), SPME, headspace and tube-based thermal desorption. All of these options offer sample enrichment on a cryogen-free, sorbent-packed focusing trap, before injection of the analytes into the GC–MS as a narrow band of vapour for optimum sensitivity.

Additional features offered by Centri include:

- Multi-step enrichment: combining multiple extracts onto the same trap for greater sensitivity.
- Re-collection: Quantitative trapping of the split flow from any sample extraction mode on a sorbent tube, for re-analysis without needing to repeat lengthy sample extraction procedures, or archiving in a stable form.

For more on Centri, visit www.markes.com.

Results and discussion

1. Analyte recovery and sensitivity

Under EU regulation, packaging that forms a protective barrier between foodstuff and the environment must not allow more than 10 ppb of total contaminants[2] to migrate into the food itself from a non-regulated material; for example, compounds from coating or print on the outside of the packaging that migrate through the barrier.

In this study, we have used a concentration of 10 ppb (and below) for individual compounds to demonstrate the sensitivity that can be achieved using Centri. Figure 1 shows the extracted ion chromatograms (EICs) for each target compound at a concentration of 10 ppb by HS–trap with multi-step enrichment (MSE) (3 vials, 1 x 5 mL headspace extraction volume from each). *o*-Xylene and styrene co-elute under the chromatography conditions used in this study; however, they can be easily distinguished by examination of EICs – amu 91 and amu 104, respectively.

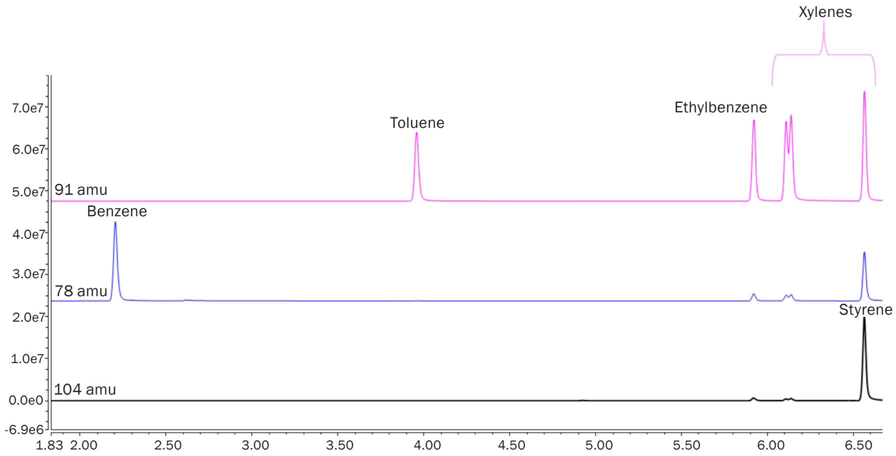
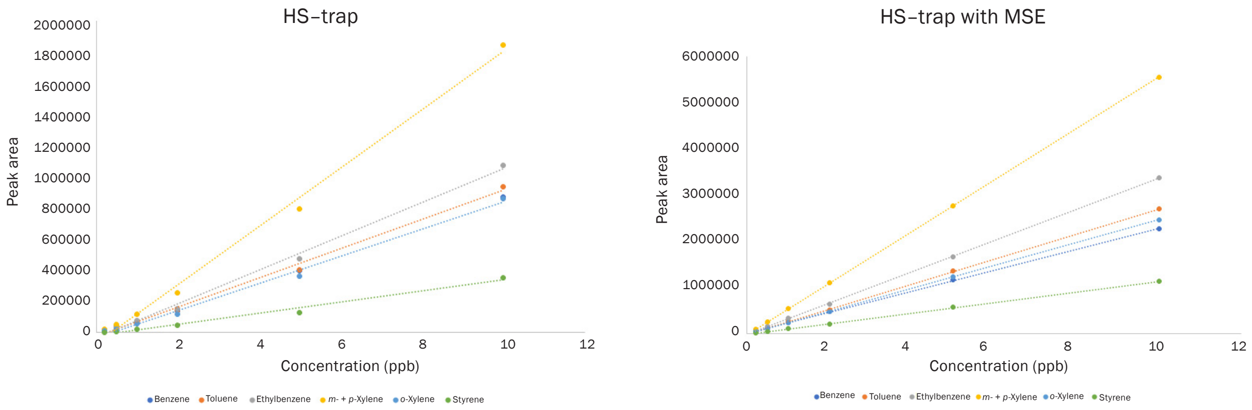


Figure 1: Extracted ion chromatograms (EICs) showing responses to target compounds at 10 ppb by headspace–trap with multi-step enrichment and GC–MS analysis.

2. Linearity

Maximum linear regression (R^2) values demonstrated the excellent linearity achieved with both HS–trap techniques (Figure 2). At the lowest level (0.2 ppb), styrene was undetectable using HS–trap, but with enrichment, the response increased, enabling detection and confident identification.

Figure 3 shows chromatographic profiles for the target analytes in 0.2–10 ppb calibration solutions obtained by HS–trap with MSE, demonstrating that target peaks are readily detectable at all levels.



Compound		**Benzene**	**Toluene**	**Ethylbenzene**	***m*- + *p*-Xylene**	***o*-Xylene**	**Styrene**
R^2	HS–trap	0.998	0.995	0.996	0.995	0.994	0.985
	HS–trap with MSE	1.000	1.000	1.000	1.000	1.000	1.000

Figure 2: Calibration plots for target compounds by HS–trap (top left) and HS–trap with multi-step enrichment (MSE) (top right), with R^2 values provided in the table.

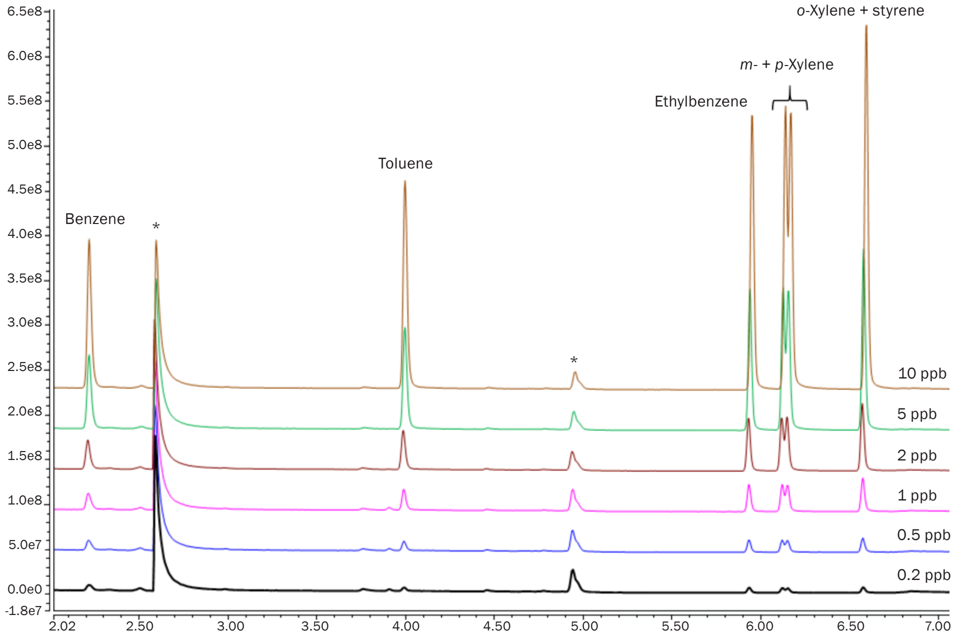


Figure 3: Total ion chromatograms showing responses to target compounds at the given standard concentration by HS–trap with MSE (* are contaminants).

3. Limit of detection (LOD)

LODs were taken to be the signal-to-noise ratio calculated from 0.2 ppb standards divided by 3. For styrene, the 1 ppb level was used, as this was the lowest measure possible with HS–trap. Separate LOD calculations were performed for the two HS–trap techniques.

LODs determined for HS–trap and HS–trap with MSE are shown in Table 1.

This confirms the expected improvement in sensitivity, where LODs for HS–trap with MSE are approximately four times lower than those achieved with HS–trap.

Compound		**Benzene**	**Toluene**	**Ethylbenzene**	***m*- + *p*-Xylene**	***o*-Xylene**	**Styrene**
LODs (ppb)	HS–trap	0.028	0.047	0.031	0.083	0.086	0.405 (1 ppb value used)
	HS–trap with MSE	0.007	0.018	0.005	0.004	0.022	0.059

Table 1: Limits of detection (LODs) calculated for BTEX-styrene compounds analysed by HS–trap and HS–trap with MSE.

4. Reproducibility

Figure 4 highlights the excellent reproducibility of results achieved when 10 ppb BTEX-styrene standards are analysed using the different HS–trap techniques (n = 6 in both cases). The relative standard deviations (RSDs) for all compounds are below 5%, indicating that the analytical methods are both robust and reliable for sample analysis.

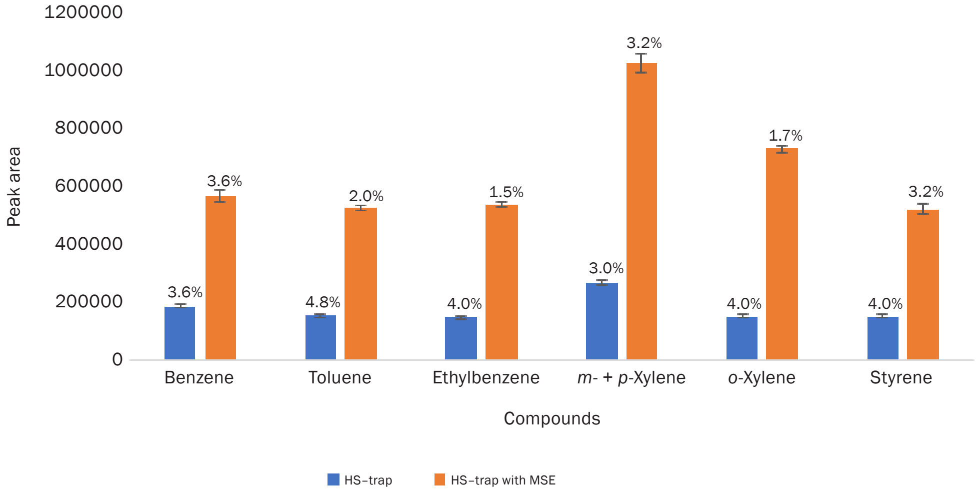


Figure 4: Reproducibility of response of target compounds at 10 ppb by HS–trap and HS–trap with MSE, with error bars indicating the standard deviations (n = 6 in both cases).

5. Food and beverage sample analysis

Three replicates were performed for each sample type, using both HS–trap and HS–trap with multi-step enrichment (MSE).

The presence of target compounds in each sample is given in Table 2, and a representative tomato paste sample is shown in Figure 5. Targets were detected more often in foodstuffs when MSE was used, demonstrating the enhancement in sensitivity achieved with this technique. Benzene was never observed; however, toluene, ethylbenzene, xylene and styrene were all detected in at least one food type.

Compound concentrations in the HS–trap with MSE analyses were calculated using the calibration curves produced in Figure 2, taking the mean peak areas of the three replicates for each sample type. The mean concentrations calculated for the target compounds detected in each sample are shown in Table 3. The most significant finding was >1 ppb concentration of toluene in the tomato paste (making up one-tenth of the maximum level of total contaminants stated in the EU regulations), which also contained the highest concentrations of xylenes and styrene compared to the other samples. The only other case of a target compound being detected at >0.1 ppb was toluene in the orange juice sample.

Levels of BTEX-styrene compounds were lowest overall in coffee. In order to ensure samples matched the product experienced by consumers, the coffee was prepared according to manufacturer instructions, by dissolving the coffee granules in near-boiling water. As a result, each 8 mL aliquot of sample contained only 72 mg of coffee granules that were exposed to the original granule packaging. Therefore, we may not expect significant amounts of BTEX-styrene from the original packaging to be present in the final sample. Target compounds detected in the coffee samples likely originate from the disposable cups and lids used to hold samples after preparation with boiling water. Of note, the most abundant target compound identified in coffee was styrene. It has previously been shown[3] that disposable polystyrene lids such as those used here emit styrene when exposed to heat, and so this is a likely explanation for the styrene detected in the coffee samples.

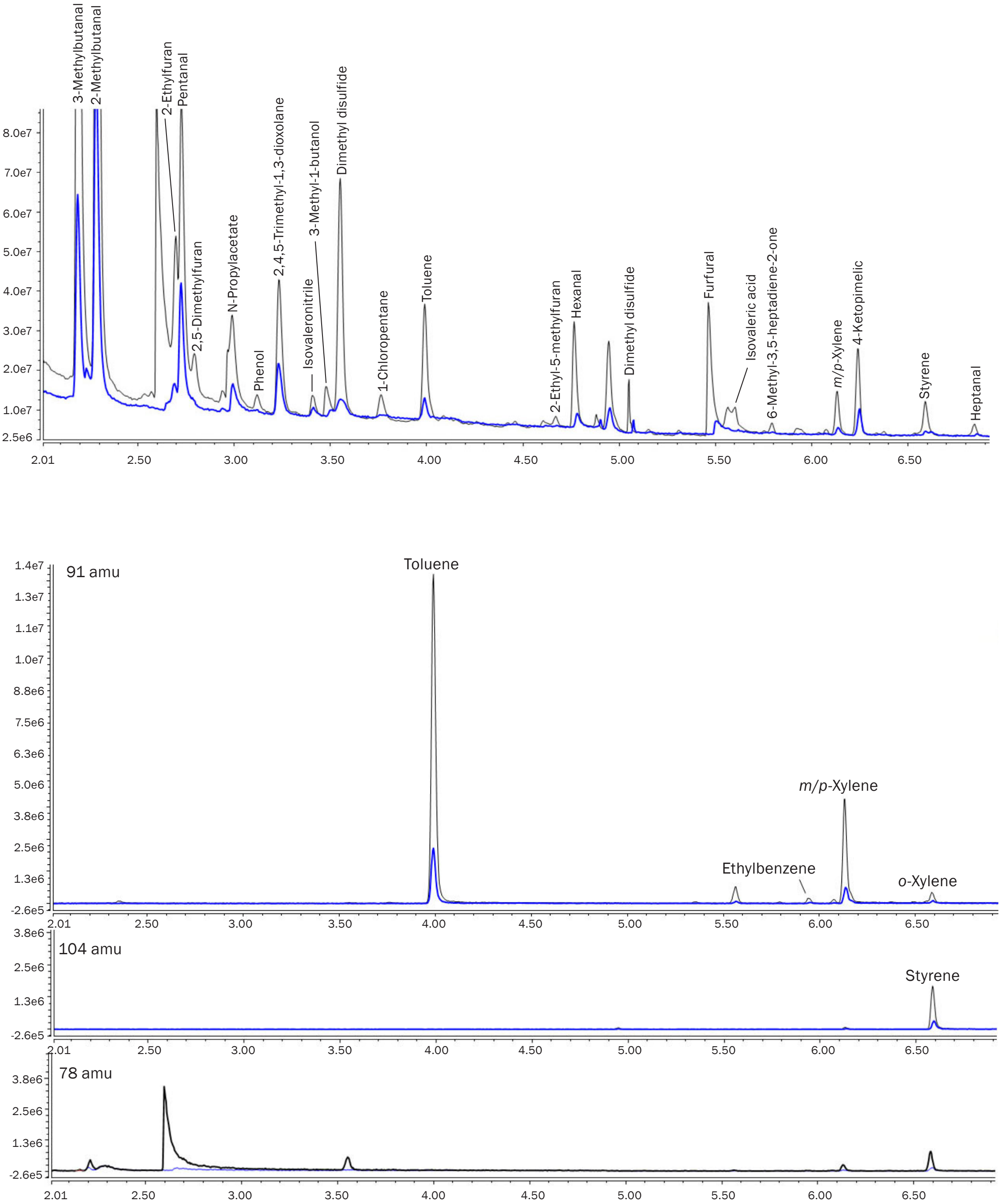


Figure 5: Chromatograms for a tomato paste sample analysed by HS–trap (blue) and HS–trap with MSE (black). In the top panel, the total ion chromatogram (TIC) shows target compounds in the context of other aroma compounds found in tomato paste. In the bottom three panels, EICs of the indicated amu values highlight BTEX-styrene compounds. Benzene would appear in the 78 amu chromatogram at retention time 2.25 but is not detected.

Sample type	HS–trap					
	Benzene	Toluene	Ethylbenzene	*m*- + *p*-Xylene	o-Xylene	Styrene
Tomato paste	X	✓	X	✓	X	✓
Orange juice	X	✓	✓	✓	✓	X
Coffee	X	✓	X	X	X	✓

Sample type	HS–trap with MSE					
	Benzene	Toluene	Ethylbenzene	*m*- + *p*-Xylene	o-Xylene	Styrene
Tomato paste	X	✓	✓	✓	✓	✓
Orange juice	X	✓	✓	✓	✓	✓
Coffee	X	✓	✓	✓	X	✓

Table 2: Sensitivity of HS–trap sampling to target compounds in food and beverages (top) and with multi-step enrichment (MSE) (bottom). A tick indicates that the compound was observed while a red cross indicates that it could not be detected.

Sample type	Concentration (ppb)					
	Benzene	Toluene	Ethylbenzene	*m*- + *p*-Xylene	o-Xylene	Styrene
Tomato paste (n = 3)	0	1.21	0.01	0.18	0.05	0.34
Orange juice (n = 3)	0	0.42	0.04	0.05	0.1	0.15
Coffee (n = 3)	0	0.04	0.02	0.01	0	0.13

Table 3: Mean concentration of target compounds detected in foodstuffs by HS–trap with MSE.

Conclusions

This study demonstrates the advantages of combining traditional HS extraction with cryogen-free trapping on Centri and HS–trap with multi-step enrichment (using multiple vials) in an important real-world application. Using multi-step enrichment (MSE), the reproducibility and linearity of response to known concentrations of BTEX-styrene matched the excellent results achieved with a single-vial extraction, indicating that the enrichment approach is suitable for quantitative analysis. When applied to real food and beverage samples, HS–trap with MSE provided striking improvements to sensitivity, allowing lower LODs to be achieved (approximately four times lower than by HS–trap). Each target compound, with the exception of benzene, was detected in at least one sample type, demonstrating the value of screening for BTEX-styrene contamination, which can pose a risk to human health. Automation with Centri ensured high sample throughput with minimal hands-on operator time, providing excellent reproducibility of results and saving time.

References

1. Markes Application Note 252.
2. https://ec.europa.eu/food/safety/chemical_safety/food_contact_materials/legislation_en
3. M. Marć and B. Zabiegała, *Microchem. J.*, 2017, 133: 496–505.

Applications were performed under the stated analytical conditions. Operation under different conditions, or with incompatible sample matrices, may impact the performance shown.