

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/221752984>

# Ambient Ionization–Accurate Mass Spectrometry (AMI–AMS) for the Identification of Nonvisible Set-off in Food–Contact Materials

ARTICLE in JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · FEBRUARY 2012

Impact Factor: 2.91 · DOI: 10.1021/jf204456z · Source: PubMed

CITATIONS

13

READS

26

## 3 AUTHORS:



[Karim Bentayeb](#)

WorldPathol Ltd. Co.

32 PUBLICATIONS 196 CITATIONS

[SEE PROFILE](#)



[Luke K Ackerman](#)

U.S. Food and Drug Administration

26 PUBLICATIONS 334 CITATIONS

[SEE PROFILE](#)



[Timothy H Begley](#)

U.S. Food and Drug Administration

80 PUBLICATIONS 1,560 CITATIONS

[SEE PROFILE](#)

# Ambient Ionization—Accurate Mass Spectrometry (AMI-AMS) for the Identification of Nonvisible Set-off in Food-Contact Materials

Karim Bentayeb,<sup>†</sup> Luke K. Ackerman,<sup>\*</sup> and Timothy H. Begley

Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, College Park, Maryland 20740, United States

## S Supporting Information

**ABSTRACT:** Set-off is the unintentional transfer of substances used in printing from the external printed surface of food packaging to the inner, food-contact surface. Ambient ionization—accurate mass spectrometry (AMI-AMS) detected and identified compounds from print set-off not visible to the human eye. AMI mass spectra from inner and outer surfaces of printed and nonprinted food packaging were compared to detect and identify nonvisible set-off components. A protocol to identify unknowns was developed using a custom open-source database of printing inks and food-packaging compounds. The protocol matched print-related food-contact surface ions with the molecular formulas of common ions, isotopes, and fragments of compounds from the database. AMI-AMS was able to detect print set-off and identify seven different compounds. Set-off on the packaging samples was confirmed using gas chromatographic—mass spectrometric (GC-MS) analysis of single-sided solvent extracts. *N*-Ethyl-2-(and 4)-methylbenzenesulfonamide, 2,4-diphenyl-4-methyl-1-(and 2)-pentene, and 2,4,7,9-tetramethyl-5-decyne-4,7-diol were present on the food-contact layer at concentrations from 0.21 to  $2.7 \pm 1.6 \mu\text{g dm}^{-2}$ , corresponding to nearly milligram per kilogram concentrations in the packaged food. Other minor set-off compounds were detected only by AMI-AMS, a fast, simple, and thorough technique to detect and identify set-off in food packaging.

**KEYWORDS:** set-off, food-contact materials, printing inks, AMI-AMS, DART

## INTRODUCTION

Printing is commonly used on food packaging not only to inform the consumer but also as a marketing tool. Printing materials contain several components that are not compatible with direct food contact and, if consumed in large quantities, could affect the health of the consumer. For this reason, the direct migration of these components through the packaging material is strictly controlled. However, set-off is a process capable of transferring print components to foods even though the permeation of these printing components through the film to the food is not likely. Set-off can be defined as the unintentional transfer of substances used in printing from the external printed surface of materials and articles intended for food packaging to the inner food-contact surface.<sup>1</sup> It can occur when the packaging is produced and stored in stacks or reels after being printed. The quantity of ink components transferred depends on the type of ink, the drying/curing procedure, the time of contact, and the pressure in the roll or stack of sheets.<sup>2</sup> Set-off is the main mechanism whereby the food could become contaminated by high molecular weight substances (>1000 Da), as diffusion of such compounds through the substrate is considered to be negligible.<sup>1</sup>

Currently, the printing industry minimizes the set-off phenomenon by reducing the use of solvents, using ultraviolet light or electron beams to polymerize prints, applying a clear varnish to protect the printed surface, and using anti-set-off powders to reduce the friction in stacks and rolls. These spray powders often contain silica or starch with a particle diameter slightly greater than the printed ink film thickness.<sup>2</sup> Once the food packaging is produced, set-off is typically controlled by visual quality control. Any batches of food packaging showing visibly detectable set-off are rejected for use. The lack of reliable

and reproducible quality controls led to the development of an optical method using a Freeman and Foster lamp, which proved to be useful for detecting nonvisible set-off of certain pigments and dyes, but not identification of the components.<sup>1</sup>

There are few studies of set-off occurrence in retail food packaging. Johns et al. detected set-off while studying the possible migration routes of model substances' transfer from cartonboard packaging stored in stacks.<sup>3</sup> Aurela et al. detected set-off of phthalates in sugar packaging<sup>4</sup> and, recently, Jung et al. detected set-off evidence after the storage of yogurt cups raised the concentrations of photoinitiators in yogurt. They demonstrated that migration of inks did not occur by permeation, but mainly by set-off processes.<sup>5</sup> Rapid detection of set-off may help to prevent large-scale food recalls.

Ambient ionization techniques (AMI) such as direct analysis in real time (DART) are ionization sources for mass spectrometry that permit the rapid and direct measurement of compounds present on the surface of solid materials. In DART, heated and excited helium desorbs and ionizes molecules on solid surfaces. The protonated/deprotonated molecular ion of the compound is commonly formed. A rapid acquisition of <20 s produces a composite spectrum containing ions of several compounds belonging to the sample.<sup>6</sup>

This recently developed technique has been used in several applications in different fields, including the analysis of food-contact materials.<sup>7</sup> Previous work has focused on the rapid detection of known additives in plastic materials. The

**Received:** November 1, 2011

**Revised:** January 11, 2012

**Accepted:** January 17, 2012

**Published:** January 17, 2012

conditions for detecting additives representing the common families of compounds in plastic materials were optimized.<sup>8</sup> Efforts to detect plasticizers in plastisols<sup>9,10</sup> or toys<sup>11</sup> have also been made. Jones et al. demonstrated the suitability of DART coupled with accurate mass spectrometry (AMS) analysis to recognize the origin of ballpoint pen ink on a piece of paper, by spectral database matching.<sup>12</sup> Morlock et al. demonstrated the ability of DART-MS to confirm the presence of ITX (an ink photoinitiator) on thin layer chromatography plaques.<sup>13</sup> To our knowledge, ambient ionization mass spectrometry (AMS) has not been evaluated as a technique to detect and identify unknown, nonvisible contamination of packaging.

The aim of this work is to test the capability of AMI-AMS to detect and identify nonvisible set-off in food packaging. Because set-off is a contamination of the packaging that occurs on the surface, analyses of single-sided solvent extracts were performed for comparison purposes. These analyses are time-consuming, involve significant quantities of solvents, and are not convenient for rapid or high-throughput testing. AMI-AMS is a good candidate for screening of packaging set-off. No sample treatment is required, acquisitions require seconds, and automation is possible. Also, AMI-AMS is not restricted to GC- or LC-amenable compounds or compounds with optical properties.

In this study, an AMI-AMS method to detect set-off on packaging was developed and compared to conventional GC-MS analysis of single-sided solvent extracts. By comparing the printed and nonprinted samples of the same packaging, the likely occurrence of set-off was rapidly detected. Comparisons of identified elemental formula with print and packaging compound databases identified set-off compounds, which were corroborated by analysis of standards and GC-MS results.

## MATERIALS AND METHODS

**Chemicals.** Methanol (Optima LC-MS, 67-56-1) and methylene chloride (GC Resolv, 75-09-2) were acquired from Fisher Scientific Co. (Pittsburgh, PA). Poly(ethylene glycol) (PEG) 600 (25322-68-3) and PEG 1000 (25322-68-3) were provided by Chem Service (West Chester, PA). Compressed nitrogen and compressed helium (99.999%) were acquired from Airgas (Hyattsville, MD). A mixture of *N*-ethyl-2-methylbenzenesulfonamide (*ortho*, 1077-56-1) and *N*-ethyl-4-methyl-benzenesulfonamide (*para*, 80-39-7) was purchased from Scientific Polymer Products, Inc. (Ontario, NY). 2,4-Diphenyl-4-methyl-1-pentene (97%, 6362-80-7), 2,4,7,9-tetramethyl-5-decylne-4,7-diol (98%, 126-86-3), benzoic acid (99%, 65-85-0), 5-ethylidene-bicyclo(2.2.1)hept-2-ene (50% ethylene, 25038-36-2), hexamethylene-tetramine (99%, 100-97-0), cyclohexyl methacrylate (97%, 101-43-9), and 1-((2-aminoethyl)amino)-2-propanol (123-84-2) were provided by Sigma-Aldrich (St. Louis, MO).

**Packaging Materials.** A multilayered flexible printed food-contact material, previously held under stacked conditions capable of generating set-off, was provided by a major food manufacturer in the United States. It consisted of at least three layers: inner, food-contact low-density polyethylene (LDPE), and aluminum and paper (printed outer surface). The same material without printing was also provided for comparison purposes.

**AMI-AMS.** AMI-AMS experiments were performed using a time-of-flight (TOF) MS JEOL AccuTOF (Peabody, MA) and DART-SVP ion source from IonSense (Saugus, MA). Standard conditions included DART to MS orifice of 1.0 cm and DART to sample distance of 2–3 mm. The helium flow rate was 1.0 L min<sup>-1</sup>, the temperature was set at 500 °C, and the DART exit grid voltage was at 530 V. Experiments were performed in positive and negative modes. The MS orifice 1 voltage was set at 30 V (protonated ions) and 60 V (fragments), and the mass sensitive entrance quadrupole lens voltage (peak voltage) was set as 600 V. The AccuTOF ring lens voltage was 5 V, orifice 2 voltage,

5 V, and orifice 1 temperature, 105 °C. The TOF was calibrated using PEG 600/1000 with mean molecular weights of 600 and 1000 Da and tuned to achieve a mass resolution above 5000 with maximum error of <5 mDa across a range from *m/z* 59 to 899. To extend the mass range to lower masses, common low mass (*m/z* 36–89) ambient ions were included in mass axis calibrations.<sup>6</sup>

Samples were acquired by cutting a small (~6 cm<sup>2</sup>) packaging area and folding it in such a way that only the desired surface under study (inner or outer) entered the helium stream. Using tweezers, the folded sample was held slightly off-center of the helium beam about 2–3 mm after the DART exit. Samples' mass spectra were acquired immediately preceded by the acquisition of PEG600/1000 to perform an internal calibration in every acquisition.

**Determination of Set-off Compounds from Spectra.** The mass spectra of the inner and outer surfaces of the printed and the nonprinted materials were acquired. Once collected, the spectrum of the food-contact surface of the nonprinted material was subtracted from the spectrum of the food-contact surface of the printed material (Boolean subtraction). The mass threshold was 5 mDa. Following this background material subtraction, the ions present only on the inner surface of the printed material were searched for in the corresponding spectra of the outer surface (printed). Each ion found in both the inner and outer printed surface but not the raw (nonprinted) background material was considered as set-off.

Set-off ions were tentatively identified using a set of programs contained in the software Mass Spec Tools of ChemSW, Inc. (Fairfield, CA). All ions above 4000 counts were searched for in the in-laboratory list of food packaging and printing ink compounds and their molecular formula using the program SearchFromList assuming some standard ionization forms and a 5 mDa mass error threshold. In positive ion mode, the following ionization forms were checked: [M + H]<sup>+</sup>, [M + H<sub>3</sub>O]<sup>+</sup>, and [M + NH<sub>4</sub>]<sup>+</sup>, forming dimers [2 × M + H]<sup>+</sup>, [2 × M + H<sub>3</sub>O]<sup>+</sup>, [2 × M + NH<sub>4</sub>]<sup>+</sup>, [M – H<sub>2</sub>O + H]<sup>+</sup>. In negative mode the ionization pattern was [M – H]<sup>-</sup>. From this accurate mass list match, a tentative assignment was made. Next, the inner surface subtracted spectra were searched for an ion matching the second most abundant isotope of every tentatively identified set-off ion (although some were likely present in too-low abundance). Finally, spectra of the packaging under higher orifice 1 voltage conditions (more fragmentation) were searched for fragment ions that were also present in the tentatively identified compound's standard spectra (collected under identical conditions).

**ATR-FTIR Experiments.** Attenuated total reflectance (ATR) Fourier transform infrared (FTIR) spectroscopy was used to check the polymer layer in the inner and outer surfaces of the packaging using a Nicolet 6700 FT-IR spectrometer with a Smart Performer Sampling Accessory provided by Thermo Fisher Scientific Inc. (Rockville, MD). A germanium crystal was used to accommodate the films. Thirty-two spectra were registered and averaged, and the wavelength range covered was from 4000 to 650 cm<sup>-1</sup>. A background spectrum was collected before each sample. Finally, the obtained spectra were matched using the library HR Hummel Polymer and Additives (version 2005).

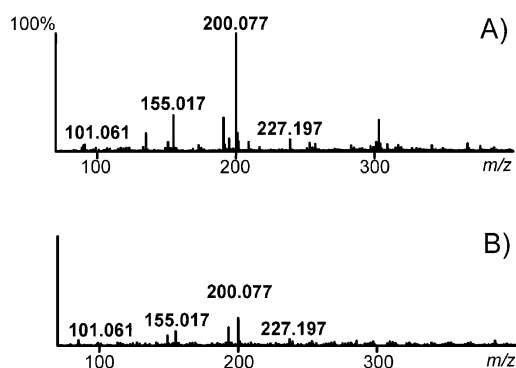
**GC-MS Confirmatory Experiments.** Single-sided extractions were performed separately on both the food-contact surface and the outer printed surface, using single-sided extraction/migration vessels provided by DURAN Group GmbH (Wertheim, Germany). Packaging surfaces (0.44 dm<sup>2</sup>) were extracted with methylene chloride (50 mL) at 21 °C for 30 min in triplicate, with corresponding method blanks.

Extracts were evaporated by gentle nitrogen flow to approximately 1 mL and analyzed by GC-MS using an Agilent 6890N gas chromatograph coupled to a 5973Network mass spectrometer (Palo Alto, CA). A HP-5MS UI capillary column (30 m × 0.25 mm × 0.25 μm film, Agilent, Palo Alto, CA) was used; a constant flow of 1.0 mL min<sup>-1</sup> helium was used as carrier. The oven ramp was as follows: 3 min at 50 °C, followed by a 10 °C min<sup>-1</sup> ramp to 295 °C and a 2.5 min hold. Run time was 30 min. Injection (1 μL) was performed in splitless mode for 1 min. Solvent delay was experimentally set at 3 min. These GC conditions were able to resolve all components of the Grob mixture. Interface, source, and quadrupole temperatures were 290, 230,

and 150 °C, respectively. GC-MS scan range was  $m/z$  50–550 using electron impact (EI) ionization after tuning with perfluorotributylamine (PFTBA). External calibration was performed to quantify the set-off compounds detected by GC-MS in the single-sided extracts. Quantification was performed in SIM mode, acquiring two different ions for each compound of interest, each with a dwell time of 100 ms. The limits of detection (LOD) were determined by injecting diluted standards. The least concentrated standard yielding a signal/noise ratio >3 was considered as the LOD.

## RESULTS AND DISCUSSION

**Detection of Set-off.** Composite, background-subtracted, and centroided AMI-AMS spectra of packaging samples were further background-subtracted of the ions also present in the never-printed packaging spectra, regardless of their intensity. The resulting inner surface spectra contained around 100 ions. Because set-off compounds on the inner (food-contact) surface come from the printing ink, the remaining ions were searched across each composite, background-subtracted, and centroided spectrum of the printed outer surface (Figure 1). A total of 25



**Figure 1.** Detection of set-off compounds by AMI-AMS: ions common to both the (A) outer, printed surface (yellow subsample), and (B) inner surface, background-subtracted.

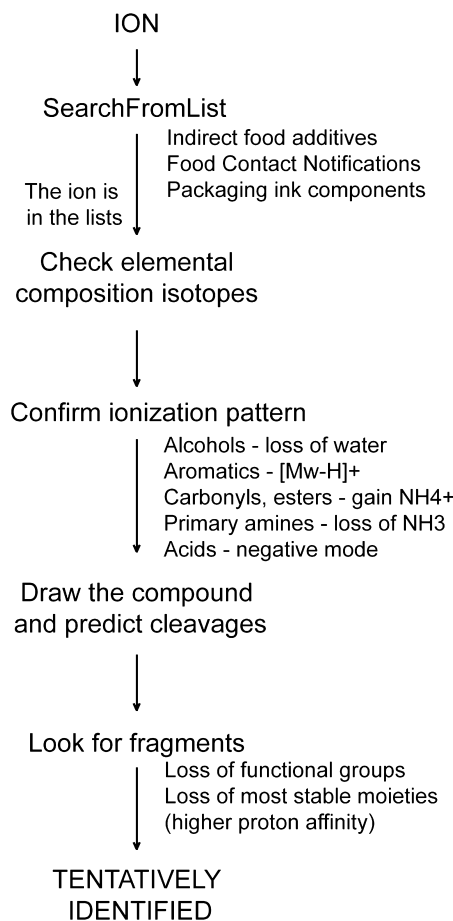
ions common to both printed and background-subtracted inner surfaces were classified as set-off related. These set-off ions were consistently detected in several subsamples of each piece of packaging, pointing to a homogenized presence of set-off across the food contact surface of the samples.

**Identification Protocol.** By combining accurate masses, elemental limits, the nitrogen rule, and the relative isotopic abundances, a unique molecular formula can often be assigned for singly charged ions of compounds with a molecular weight below 800 Da. These formulas can then be corrected to neutral molecular formulas and searched against lists of probable compounds. Mass accuracy was controlled and kept within 5 mDa, although observed mass errors on the calibrants were often lower (<2 mDa). It was observed that mass errors on standards increased dramatically when ion intensities dropped below certain values, and errors increased slowly with detector saturation and mass peak skewing above certain intensities. Therefore, ion intensity was checked in every spectrum to help ensure the 5 mDa accuracy was met. This led to a reliable acquisition mass range from  $m/z$  59 to 899, primarily dictated by PEG ion abundance/mass accuracy. Sample ions acquired out of these ranges were not considered to be accurate enough for identification purposes.

The identification of the print packaging related unknowns was addressed by using the SearchFromList program of Mass Spec Tools. This software searches every ion detected in a

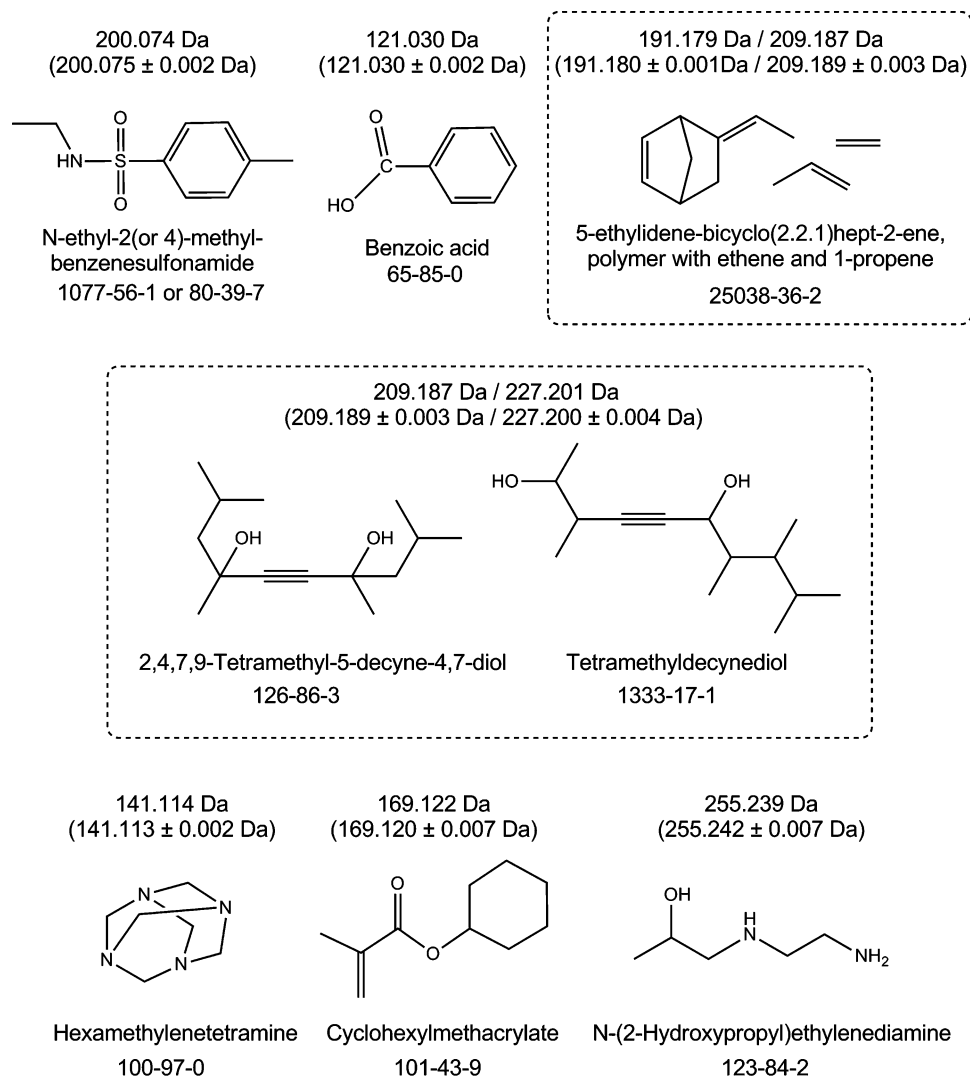
spectrum against a list of target molecular formula created by the user, comparing the mass observed with the mass of a molecular formula of the list (taking into account the common ionization patterns observed in chemical ionization) and provides a list of matched compounds (within 5 mDa mass error). We prepared a within-laboratory compound list compiled from several publicly available lists of print packaging related compounds (see Table S-1 of the Supporting Information). None of the databases consulted contained the molecular formula of the compounds, so an open-source macro routine (ChemCell, Collaborative Drug Discovery, Inc., Burlingame, CA) used CAS Registry Numbers to retrieve the molecular formula from the Chemical Structure Lookup Service of the National Cancer Institute.<sup>14</sup> In total, 2257 molecular formulas were included in the in-laboratory database (see Table S-1 of the Supporting Information).

When the SearchFromList program was applied to the acquired spectra, up to 70% of the ions were matched to one or more compounds in the database. During method development, initial SearchFromList queries of standards' spectra yielded a high number of different molecular formulas in the customized list for every ion of the known standards. Thus, the presence of several isomers in the customized list, together with the potential combinations of adducts and dimers, led to multiple positive matches, which required further selection. To minimize false positives and avoid false negatives, a decision tree was used to assess the reliability of and identify the detected set-off ions (Figure 2).



**Figure 2.** Protocol to tentatively identify "known-unknown" set-off compounds detected by AMI-AMS.





**Figure 3.** Set-off compounds tentatively identified by AMI-AMS, theoretical and observed (parentheses) masses, and CAS Registry Numbers.

Once the set-off ions were matched to prospective print compounds by the SearchFromList program, the first step was to check the detected isotopes and their relative intensities using the program Elemental Composition of Mass Spec Tools. Once the isotope's relative abundance matched to within 0.1% and mass to within 5 mDa, the empirical formula of the compound was considered to be known. The set-off ions matched to print related compounds were further screened against probable ionization patterns according to their structure; for example, the ionization pattern  $[M - H_2O + H]^+$  should match with an alcohol and not with the isomeric ether. Finally, the candidate structures were drawn, and the fragments derived from likely cleavages (ChemBioDraw Ultra, CambridgeSoft, Cambridge, MA) were searched for in the spectrum acquired at 60 V orifice 1 voltage. If one predicted fragment was found, then the compound was considered to be "tentatively identified".

This identification protocol was applied and tested with a group of 41 print and polymer related standards previously acquired. A large range of chemicals were studied using AMI-AMS, including compounds that cannot be determined easily by GC-MS, such as acids or large (>800 Da) antioxidants. This test group is listed in Table S-2 of the Supporting Information. The SearchFromList program matched the major ions in all 41

compounds AMI-AMS spectra. However, when the Elemental Composition program was run, the actual empirical formula was not matched as the first choice for nine compounds (20%). Six of the nine standards' ions were too low in abundance for an accurate mass assignment of the second most abundant isotopic ion. In the other three cases, an empirical formula containing sulfur was erroneously matched as first choice. Because no sulfur-containing isobaric compounds were present in the lists of potential compounds used in the SearchFromList program, the combination of both approaches (SearchFromList and Elemental Composition matching) ultimately led to the correct identification of all of the standards.

The previous protocol was applied to the 25 ions considered as potential set-off compounds, leading to the tentative identification of 7 compounds using 20 of these ions. Figure 3 shows the main features of the 7 tentatively classified set-off compounds. As can be seen, the observed mass matched the theoretical masses (0.2–2.9 mDa) and were quite stable ( $n = 3$ ). Five set-off related ions in the subtracted spectra did not match any of the compounds in the lists ( $m/z$  253.206, 157.115, 156.019, 256.217, and 185.144). Comparisons were made to GC identified set-off compounds and EI-MS fragments, but no matches were found, and no further identification was attempted.

Of the tentatively classified set-off compounds, most of the compounds were clearly ink related. *N*-Ethyl-2(or 4)-methylbenzenesulfonamide is generally used as a plasticizer in printing inks.<sup>15</sup> Whereas benzoic acid is used in printing inks, the ion at  $m/z$  121.029 probably came from an additive that contains benzoic acid in the structure, as occurs in some commonly used dyes.

The ions at  $m/z$  191.179 and 209.187 were identified as the basic unit of a bicyclodiene polymer documented as a direct additive in the packaging industry. No references relating to printing inks were found for this compound. These could be misidentified ions, or the compound was not related to set-off. On the other hand, both  $m/z$  209.187 and 227.197 also were consistent with the tetramethyldecynediol structures depicted in Figure 3. The compound 2,4,7,9-tetramethyl-5-decyne-4,7-diol (TMDD) is more often used than the tetramethyldecynediol isomer, so it was expected that these ions belong to the former TMDD. The ion at  $m/z$  227.197 corresponds to the protonated ion, whereas  $m/z$  209.187 corresponds to the loss of water from the protonated molecule. Furthermore, the ion at  $m/z$  191.179 matched a fragment of TMDD with the loss of both hydroxy groups. TMDD is used as nonionic surfactant in water-based printing inks.

The  $m/z$  255.240 ion was identified as *N*-(2-hydroxypropyl)-ethylenediamine. This compound is also a direct additive used in food packaging, but we did not find literature suggesting its use in printing. Although the  $m/z$  255.240 ion was originally classified as set-off, it is possible that this is a misclassification because of its use in bulk food packaging components. The protocol also led to the identification of  $m/z$  144.141 as hexamethylenetetramine, a curing agent for phenolic resins.

Other ions were matched with the empirical formulas of methacrylate and acrylate derivatives ( $m/z$  101.065, 169.121, and 205.103), pointing out that the ink on the outer layer of this tested package could contain or be coated with acrylic resins. These resins are widely used as vehicles in UV-cured printing inks.

**Confirmation of the Set-off Occurrence. ATR-FTIR Characterization of Inner and Outer Layers.** An alternative explanation to the presence of an ion on both inner and outer surfaces of the printed material could be that the finished material had the same polymeric layer (varnish) covering both surfaces. This possibility was rejected after the determination of polymer identities of both sides using ATR-FTIR spectroscopy. The food contact surface matched 95% with linear polyethylene, whereas the outer surface matched 78% with a poly(styrene-acrylonitrile:methyl acrylate). These results suggested that the outer print surface was covered by an acrylic resin. This type of resin is widely used in printing inks. The detection of some methacrylate and acrylate derivatives by AMI-AMS is consistent with this FTIR result.

**Single-Sided Extraction and GC-MS Analysis.** Table 1 lists the set-off compounds detected by GC-MS in the concentrated extracts. These compounds were detected on both the inner and outer surfaces of the printed material and not on the nonprinted packaging or the method blanks. Six different set-off compounds were detected using GC-MS. Five of these set-off compounds were identified using both Kovats retention index and EI-MS library spectral matching (NIST08). Using purchased standards, five were confirmed and quantified. Two new compounds (isomers) not identified by AMI-AMS analysis were detected: 2,4-diphenyl-4-methyl-1-pentene and 2,4-diphenyl-4-methyl-2(*E*)-pentene. However, AMI-AMS

detected (but did not identify) a fragment of these isomers ( $m/z$  119.086). The packaging related classification of diphenylmethylpentenes was identified through the print/packaging database as chain transfer agents used to stop polymerization.

Concentrations of the set-off compounds on the inner food-contact surface ranged from 0.2 to 2.7  $\mu\text{g dm}^{-2}$ , for a total of  $7.0 \pm 4.4 \mu\text{g dm}^{-2}$ . The area/food weight ratio in this packaging (taco seasoning pouch) is 83  $\text{dm}^2 \text{kg}^{-1}$  of food. Assuming complete mass transfer (a worst case but not unrealistic scenario), the set-off contamination would result in a concentration of  $0.95 \pm 0.59 \text{ mg kg}^{-1}$  of food.<sup>16</sup> In the case of *N*-ethyl-2(and 4)-toluenesulfonamide, the maximum transference would lead to a food concentration of  $0.36 \pm 0.22 \text{ mg kg}^{-1}$ . In comparison, in the absence of a specific migration limit, the European Food Safety Authority sets a default limit for substances not explicitly approved for food contact (printing related compounds) of 0.01  $\text{mg kg}^{-1}$  food. Further evidence for direct set-off of these compounds from technical printing ink included the isomer ratios. The relative concentration of the isomers *N*-ethyl-2-methylbenzenesulfonamide and *N*-ethyl-4-methylbenzenesulfonamide (3:2) in both printed and food-contact surfaces matched the technical standard.

Table 1 shows that approximately 1–4% of 2,4,7,9-tetramethyl-5-decyne-4,7-diol and *N*-ethylmethylbenzenesulfonamide isomers likely transferred from the printed surface to the food-contact surface. However, 21–24% of the 2,4-diphenyl-4-methylpentene isomers were likely transferred, which is much larger than previously expected set-off transfer rates. Because this polymer termination compound is not used in the manufacture of LDPE resins, it is unlikely that the exterior–interior concentration ratio is skewed by co-occurrence in LDPE. Further studies are needed to establish the accuracy and mechanistic rationale for this observation.

**AMI-AMS Performance versus Single-Sided Extraction GC-MS.** Table 1 also shows the AMI-AMS performance related to the set-off compounds identified by GC-MS. 2,4,7,9-Tetramethyl-5-decyne-4,7-diol and *N*-ethylmethylbenzenesulfonamide were also identified by AMI-AMS, as explained in previous sections. AMI-AMS detected, but did not identify, 2,4-diphenyl-4-methyl-1-pentene because a fragment and not the protonated ion was observed. All five compounds identified by GC-MS were also detected by AMI-AMS, but only four were identified by the identification protocol when the detected compound was extensively fragmented.

On the other hand, AMI-AMS analysis detected additional set-off ions associated with compounds that were not identified by GC-MS. To address these differences, standards of AMI-AMS identified compounds were purchased and analyzed by GC-MS-SIM. Estimated LODs were determined as the lowest concentration standard solution to easily generate a visible chromatographic peak. These estimated LODs ranged from 0.06 to 0.20  $\mu\text{g dm}^{-2}$ . Because three of these compounds were not detected in the extracts of the printed surface, a likely misidentification by the AMI-AMS protocol occurred. The set-off associated ions could correspond with fragments of higher molecular weight less-volatile compounds that were not detected by GC-MS. Additionally, two of the AMI-AMS tentatively identified set-off compounds are not volatile and/or were labile and, thus, were not capable of being confirmed by the GC-MS protocol.

Although AMI-AMS was able to detect most of the compounds detected by GC-MS when the AMI temperature was 250 °C, additional ions of the nonconfirmed set-off compounds

Table 1. Set-off Compounds Determined by GC-MS and AMI-AMS

name	CAS Registry No.	molecular formula	GC-MS			AMI-AMS		
			Kovats index	inner surface ( $\mu\text{g dm}^{-2}$ )	outer surface ( $\mu\text{g dm}^{-2}$ )	set-off ions ( $m/z$ )	identified?	AMI temperature ( $^{\circ}\text{C}$ ) ion intensity
2,4,7,9-tetramethyl-5-decyne-4,7-diol	126-86-3	$\text{C}_{14}\text{H}_{26}\text{O}_2$	1419	$0.21 \pm 0.07$	$5.6 \pm 2.1$	$[\text{M} + \text{H} - \text{H}_2\text{O}]^+$ (209.187) fragment (125.097)	yes	500 7601
unknown	-	-	1654	na <sup>a</sup>	na <sup>a</sup>	-	-	-
N-ethyl-2-methylbenzenesulfonamide	1077-56-1	$\text{C}_9\text{H}_{13}\text{NO}_2\text{S}$	1676	$2.7 \pm 1.6$	$216 \pm 57$	$[\text{M} + \text{H}]^+$ (200.074) isotopic ion (201.077) fragment (155.016)	yes <sup>b</sup>	250/500 74413
N-ethyl-4-methylbenzenesulfonamide	80-39-7	$\text{C}_9\text{H}_{13}\text{NO}_2\text{S}$	1734	$1.7 \pm 1.1$	$135 \pm 41$	$[\text{M} + \text{H}]^+$ (200.074) isotopic ion (201.077) fragment (155.016)	yes <sup>b</sup>	250/500 74413
2,4-diphenyl-4-methyl-1-pentene	6362-80-7	$\text{C}_{18}\text{H}_{20}$	1810	$1.4 \pm 1.0$	$6.3 \pm 1.0$	fragment (119.086)	no <sup>b</sup>	250/500 19573
2,4-diphenyl-4-methyl-2(E)-pentene	22768-22-5	$\text{C}_{18}\text{H}_{20}$	1854	$1.1 \pm 0.6^c$	$4.8 \pm 1.0^c$	fragment (119.086)	no <sup>b</sup>	250/500 19573
benzoic acid	65-85-0	$\text{C}_7\text{H}_6\text{O}_2$	1168	<LOD <sup>d</sup>	<LOD <sup>d</sup>	$[\text{M} - \text{H}]^-$ (121.029) fragment (77.039)	yes	500 14957
S-ethylidenecyclo(2.2.1) hept-2-ene polymer with ethene and 1-propene	25038-36-2	$\text{C}_{14}\text{H}_{22}$	na <sup>e</sup>	na <sup>e</sup>	na <sup>e</sup>	$[\text{M} + \text{H}]^+$ (191.179)	yes	500 7973
hexamethylenetetramine	100-97-0	$\text{C}_6\text{H}_{12}\text{N}_4$	1235	<LOD <sup>d</sup>	<LOD <sup>d</sup>	fragment (121.101) $[\text{M} + \text{H}]^+$ (141.111)	yes	500 7453
cyclohexyl methacrylate	101-43-9	$\text{C}_{10}\text{H}_{16}\text{O}_2$	1210	<LOD <sup>d</sup>	<LOD <sup>d</sup>	$[\text{M} + \text{H}]^+$ (169.121) fragment (83.086)	yes	500 6803
1-((2-aminoethyl)amino)-2-propanol	123-84-2	$\text{C}_3\text{H}_{14}\text{N}_2\text{O}$	na <sup>e</sup>	na <sup>e</sup>	na <sup>e</sup>	$[2 \times \text{M} + \text{H}_3\text{O}]^+$ (255.240) fragment (137.128)	yes	500 4622

<sup>a</sup>NIST library failed to yield a MS match. Quantification could not be performed. <sup>b</sup>No differences between the isomers could be detected by AMI-AMS. <sup>c</sup>2,4-Diphenyl-4-methyl-2(E)-pentene was quantified using the calibration curve of 2,4-diphenyl-4-methyl-1-pentene. <sup>d</sup>Not detected in the extracts (LOD benzoic acid =  $0.07 \mu\text{g dm}^{-2}$ , LOD hexamethylenetetramine =  $0.20 \mu\text{g dm}^{-2}$ , LOD cyclohexyl methacrylate =  $0.06 \mu\text{g dm}^{-2}$ ). <sup>e</sup> $10 \text{ mg L}^{-1}$  standard not detected by GC-MS.

were detected when the temperature was increased to 500 °C. From this it can be inferred that AMI-AMS covers a wider compound range than GC-MS for the detection of set-off compounds. It must also be realized that the printing/curing process can create compounds for which commercial standards are not available; therefore, positive confirmation of identity will not always be possible.

To summarize, the use of AMI-AMS was effective in quickly (<10 min) detecting the presence of nonvisible set-off in these samples of food packaging. This was achieved by comparing printed, finished packaging and nonprinted packaging spectra. The time-consuming step of identifying the set-off compounds detected was reduced to a few hours by combining the manufacturer's elemental composition software with a custom database (from open sources) of more than 2000 unique compounds with molecular formulas used in food packaging and printing inks. The whole procedure detected at least 9 set-off compounds; 7 were identified, and 3 of these 7 were ultimately detected and identified by GC-MS. The identification protocol for AMI-AMS failed in the case of the two isomers detected but not identified because only fragment ions were observed. Therefore, further research to improve the identification should focus on modifying acquisitions to consistently generate protonated molecular ions or to better distinguish fragments ions.

Clearly, AMI-AMS showed sufficient sensitivity to detect nonvisible set-off compounds, covered a wider range of compounds than GC, and was automated, faster, and used no manual sample preparation like extraction GC-MS. This application could be easily used within the printing industry, where a more inexpensive low-resolution mass spectrometer would serve to detect the known print related chemicals. In the case of food safety laboratories, the availability of nonprinted control samples is rare, so knowledge of the underlying materials of samples, standard polymer samples (or simply set-off free regions) might provide similar controls for accurate background subtraction. Finally, these experiments demonstrate the presence of previously unidentified nonvisible set-off widely distributed across the food contact surface rather than in discrete islands. This set-off would likely come from a non-visible protective varnish applied over the printed ink. As demonstrated, some of the components are capable of reaching microgram per kilogram concentrations in foods.

## ■ ASSOCIATED CONTENT

### ■ Supporting Information

Table S-1 (databases used as SearchFromList sources, updated December 2010) and Table S-2 (evaluation of the protocol of identification by AMI-AMS using food packaging standards) and additional information as noted in the text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## ■ AUTHOR INFORMATION

### Corresponding Author

\*E-mail: [Luke.Ackerman@fda.hhs.gov](mailto:Luke.Ackerman@fda.hhs.gov). Phone: 1 (240) 402 2266.

### Present Address

<sup>†</sup>Department of Analytical Chemistry, University of Zaragoza, Maria de Luna 3, 50018 Zaragoza, Spain.

### Funding

K.B. acknowledges the Departamento de Ciencia, Tecnología y Universidad of the Gobierno de Aragón (Spain), for the

fellowship provided by the program "Fomento de la Movilidad de los Investigadores" in 2011.

## Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

We acknowledge William Limm, at the Center for Food Safety and Applied Nutrition, for his support with FT-IR experiments.

## ■ REFERENCES

- (1) Bradley, E. L.; Castle, L.; Dines, T. J.; Fitzgerald, A. G.; Tunon, P. G.; Jickells, S. M.; Johns, S. M.; Layfield, E. S.; Mountfort, K. A.; Onoh, H.; Ramsay, I. A. Test method for measuring non-visible set-off from inks and lacquers on the food-contact surface of printed packaging materials. *Food Addit. Contam.* **2005**, *22*, 490–502.
- (2) Aurela, B. Food packaging inks and varnishes and chemical migration into food. In *Chemical Migration of Food Contact Materials*; Barnes, K. A., Sinclair, R. C., Watson, D. H., Eds.; Woodhead Publishing: Cambridge, U.K., 2007; pp 302–318.
- (3) Johns, S. M.; Jickells, S. M.; Read, W. A.; Gramshaw, J. W.; Castle, L. Studies on functional barriers to migration: Use of model substances to investigate transfer from paper and board packaging to food. *Dtsch. Lebensm.-Rundsch.* **1996**, *92*, 273–278.
- (4) Aurela, B.; Ohra-aho, T.; Soderhjelm, L. Migration of alkylbenzenes from packaging into food and Tenax (R). *Packag. Technol. Sci.* **2001**, *14*, 71–77.
- (5) Jung, T.; Simat, T. J.; Altkofer, W. Mass transfer ways of ultraviolet printing ink ingredients into foodstuffs. *Food Addit. Contam.* **2010**, *27*, 1040–1049.
- (6) Cody, R. B.; Laramée, J. A.; Durst, H. D. Versatile new ion source for the analysis of materials in open air under ambient conditions. *Anal. Chem.* **2005**, *77*, 2297–2302.
- (7) Harris, G. A.; Galhena, A. S.; Fernandez, F. M. Ambient sampling/ionization mass spectrometry: applications and current trends. *Anal. Chem.* **2011**, *83*, 4508–4538.
- (8) Ackerman, L. K.; Noonan, G. O.; Begley, T. H. Assessing direct analysis in real-time-mass spectrometry (DART-MS) for the rapid identification of additives in food packaging. *Food Addit. Contam.* **2009**, *26*, 1611–1618.
- (9) Ackerman, L. K.; Noonan, G. O.; Begley, T. H.; Simoneau, C.; Suman, M. Dart-Ms/Ms analysis of foamed PVC jar lids. In *57th American Society for Mass Spectrometry Conference on Mass Spectrometry*, Pennsylvania Convention Center, Philadelphia, PA, 2009.
- (10) Rothenbacher, T.; Schwack, W. Rapid identification of additives in poly(vinyl chloride) lid gaskets by direct analysis in real time ionisation and single-quadrupole mass spectrometry. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 21–29.
- (11) Rothenbacher, T.; Schwack, W. Rapid and nondestructive analysis of phthalic acid esters in toys made of poly(vinyl chloride) by direct analysis in real time single-quadrupole mass spectrometry. *Rapid Commun. Mass Spectrom.* **2009**, *23*, 2829–2835.
- (12) Jones, R. W.; Cody, R. B.; McClelland, J. F. Differentiating writing inks using direct analysis in real time mass spectrometry. *J. Forensic Sci.* **2006**, *51*, 915–918.
- (13) Morlock, G.; Schwack, W. Determination of isopropylthioxanthone (ITX) in milk, yoghurt and fat by HPTLC-FLD, HPTLC-ESI/MS and HPTLC-DART/MS. *Anal. Bioanal. Chem.* **2006**, *385*, 586–595.
- (14) National Cancer Institute (CADD Group Chemoinformatics Tools and User Services), Chemical Structure Lookup Service; <http://cactus.nci.nih.gov/chemical/structure/>.
- (15) Forrest, M. *Coatings and Inks for Food Contact Materials*; Smithers Rapra Technology: 2007; ISBN:9781847350794.
- (16) Biedermann, M.; Grob, K. Is recycled newspaper suitable for food contact materials? Technical grade mineral oils from printing inks. *Eur. Food Res. Technol.* **2010**, *230*, 785–796.