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Analysis of trace amounts of adulterants found in powders/ supplements utilizing Raman spectroscopy coupled to direct analyte-probed nanoextraction-nanospray ionization-mass spectrometry†

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Abstract

In the United States, all food products have to be regulated to inform the consumers of the ingredients contained within. Some ingredients are not included on the label and yet are still found in the products. Presented is a Raman imaging technique for rapid, nondestructive, and spatially relevant localization of adulterants in powders. Raman spectroscopy followed by direct analyte-probed nanoextraction coupled to nanospray ionization-mass spectrometry allows rapid determination of the presence of each adulterant, leading to positive identifications such as melamine. The location and identification of these trace particles can then be extracted using a nanomanipulator. The nanomanipulation technique uses a solvent filled capillary tip which can be positioned on the particle of interest. Direct mass spectrometric analysis *via* nanospray of the particulate of interest eliminates time consuming chromatographic techniques prior to mass spectrometry analysis. This coupled technique combines rapid Raman spectroscopy techniques with direct mass spectrometry to confirm the presence of an adulterant. This technique was applied to an FDA supplied test sample, in which sibutramine, phenolphthalein, and melamine were confirmed to be present.

Introduction

Within the past 35 years the obesity rates in the United States for adults have more than doubled, with an average person being 24 pounds heavier today than in 1960.¹ In light of this information, many people are becoming more health conscious and striving for a healthier lifestyle, which typically involves a proper diet and regular exercise. There has also been an increase in “healthy weight loss” products that are easily available on the market for consumption. These products include herbal medicines, dietary supplements, weight-loss supplements and powders, which are being advertised as healthy or “all-natural”. However,

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numerous products have previously been tested and found to contain many types of contaminants such as steroids, synthetic and non-synthetic drugs, and other synthetic and non-synthetic adulterants,^{2–12} which could be harmful to a person. Intentional or accidental failure to include adulterants in the ingredient labels often results in unregulated consumption of harmful or toxic ingredients in an attempt to elicit a desired weight-loss effect. The Food and Drug Administration (FDA) regulates these products under foodstuff and thus they are not evaluated as a drug would be before public release.¹¹ Many adulterants such as melamine, a nitrogen rich chemical, are a growing public health concern. Although the toxicity of melamine and its analogues is well accounted for and a breadth of analytical techniques have been established,¹³ there is still a need for further methods of investigation, for all adulterants.

Previously, liquid chromatography-mass spectrometry techniques were the primary methods of analysis of these substances. Synthetic adulterants in herbal weight-reducing dietary supplements were analyzed using high performance liquid chromatography/electrospray ionization mass spectrometry (HPLC/ESI-MS),⁴ synthetic drugs were found in botanical dietary supplements using a QTRAP LCMS/MS system,¹² and LC-ESI-MS-MS was used for the detection of synthetic adulterants in herbal remedies.⁵ More recently, in the past few years, some more techniques have been used to analyse these powders and supplements, for example, the use of Fourier transform infrared (FTIR) spectroscopy with attenuated total reflectance (ATR) for the detection of known adulterants within dietary ingredients⁷ and toxic metals within selected plants and supplements were determined using inductively coupled plasma with mass spectrometry (ICP-MS).⁸ A portable ion mobility spectrometer has also been used to screen supplements for specific adulterants,⁹ and for analyzing the content of herbal supplements, X-ray powder diffractometry and liquid chromatography were used.¹⁰ The Food Safety and Inspection Service (FSIS) and the FDA of the United States Department of Health and Human Services and Department of Agriculture have both established gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) techniques for the determination of melamine and other adulterants in foodstuffs.¹⁴ Typical GC or LC analysis has been further amended *via* initial microextractive or targeted isolation methods. Separatory GC methods have been developed for the isolation of melamine and its analogues simultaneously providing qualitative and quantitative data *via* GC-MS/MS.¹⁵ Novel solid phase microextraction methods have been developed, specifically using zirconia hollow fiber sorptive microextraction and subsequent analysis *via* GC-MS.¹⁶ Molecularly imprinted polymers (MIPs) in microsphere form extract melamine for subsequent analysis by GC-MS, which has also been established as selective for melamine in milk and feedstocks.¹⁷

Currently post-consumption and food product analyses are the two types of testing methods. Post-consumption analysis is contingent on sampling excreted biological waste, primarily *via* urinalysis.^{18,19} Post-consumption has the disadvantage that the chemical has already been ingested and consequently biological damage is ongoing. Laboratory evaluations examine the efficacy of established procedures, often using tandem chromatographic-mass spectrometric techniques, by blind sample determination.²⁰ Ongoing tests continue to target the proficiency of these laboratories in their efficacy in applying analytical techniques to melamine determination²¹ and other adulterants. Targeted food product analysis techniques

seek to become accepted and effective as screening techniques to prevent the ingestion of unknown adulterants.

Since 2004, new techniques and instrumentation have been developed to avoid time consuming chromatographic techniques and adopt rapid direct analysis *via* ambient ionization MS. Ambient ionization mass spectrometry allows for the equipment to be used under ambient conditions, minimal sample preparation and access, and immediate results.^{22,23} Techniques that emerged from the developed ambient ionization techniques include desorption electrospray ionization (DESI), direct analysis in real time (DART), electrospray-assisted laser desorption/ionization (ELDI), paper-spray ionization (PI), *etc.*²³ These new techniques have also been established and demonstrated with several types of samples including: explosives, illicit drugs, pharmaceutical drugs and more.²²

Raman and other spectroscopy techniques are well suited for melamine, analogue, and other adulterant identification, especially when used as an imaging technique, as spatially relevant data can help locate individual particulates. Raman spectroscopy has been applied to liquid and powdered milk samples,^{24,25} including imaging techniques, with developed algorithms to approximate the concentration.²⁶ Surface-enhanced Raman scattering (SERS) techniques have also been applied to melamine and its analogues using gold nanoparticles (NPs).^{27–29} SERS microprobes have been further applied to obtain point source spectra and subsequent removal of the surfactant molecules *via* switching of the applied potential.³⁰ Macro-scale Raman imaging detected multiple adulterants, with 0.25 mm resolution.³¹ Fluorescence based methods observe competitive absorption between melamine and gold NPs with fluorescein attached.^{32,33} Fluorescence sensors based upon cucurbit[*n*]uril, specifically CB7, detect melamine in milk products.³⁴ Near-infrared and mid-infrared imaging provided spatial distributions of additive components in dairy products.^{35,36} Fourier transform infrared-attenuated total reflectance (FTIR-ATR) methods have been used in screening raw materials destined for supplements.³⁶ Spectroscopy based techniques are overall rapid, nondestructive, and specific for melamine and its analogue contaminants.¹⁴

Established here is a preliminary method of Raman imaging an adulterated powder sample to localize specific contaminants. This serves as a rapid screening method providing preliminary results and spatially relevant localization. Subsequent direct analyte-probed nanoextraction coupled to nanospray ionization-mass spectrometry (DAPNe-NSI-MS) allows for the pinpoint extraction of localized contaminant particles for confirmatory analysis. DAPNe-NSI-MS has been established as an extractive ambient ionization technique for: (1) drug residues from latent fingerprints,³⁷ (2) extraction of trace analytes for fiber analysis,³⁸ (3) forensic analysis of illicit drugs from electrostatic lifts,³⁹ (4) document and ink analysis,⁴⁰ and (5) biological cancer single cell/single organelle analysis.^{41,42} This series of analytical techniques serves as a rapid screening method for a specific contaminant *via* Raman spectroscopy with positive identification providing localization for precise extraction *via* the DAPNe-NSI-MS method. The combination of these techniques could be quite accommodating in a situation in which the sample is limited. This establishes DAPNe-NSI-MS as a non-targeted technique allowing for an all-inclusive particle search resulting in high selectivity, without large amounts of sample and time requirements of LC-MS or GC-MS.

Materials and methods

Reagents and solvent preparation

The utilized chemicals and solvents include Optima LC/MS grade acetonitrile (Fischer Scientific; Fair Lawn, NJ), ACS grade formic acid (Acros Organics; New Jersey, USA), glacial acetic acid (Sigma-Aldrich, St. Louis, MO) and millipore water with 18 M Ω resistivity which was obtained from an Aqua Solutions water purification system (Aqua Solutions, Inc.; Jasper, GA). Two solvents were prepared, the first solvent was composed of a 1 : 1 (v/v) solution of acetonitrile : water and 0.1% of formic acid; the second solvent was composed of a 70 : 30 (v/v) solution of methanol : water and 1% of acetic acid.

Samples

Samples A and B, an “all-natural” weight-loss supplement containing sibutramine and phenolphthalein, and a wheat flour product containing melamine were obtained as blind test samples from the FDA. Sample A is in light beige/tan color while sample B is in light beige/off-white color both with specks of dark and crystalline pieces as shown in Fig. S1.†

Instrumentation

The instrumentation workstation consists of a TE2000 inverted microscope (Nikon, Melville, NJ, USA) with 10 $\times$, 40 $\times$, 50 $\times$ and 100 $\times$ objectives. This microscope was fitted with a Nikon CCD camera and NSI Elements software (Nikon; Melville, NY) which permitted the viewing and image capture of the sample morphology, particle sizes and colors through the use of a differential interference contrast (DIC) polarizer. Fluorescence observations were made using an Intensilight C-HGFI fluorescence source (Nikon; Melville, NJ). An iHR550 Raman Imaging Spectrometer with a Synapse detector (HORIBA Scientific; Edison, NJ) and a 785 nm laser source (Innovative Photonic Solutions, Fat Boy Laser Module, Monmouth Junction, NJ) is mounted in conjunction with the inverted microscope. Approximately 5.0 mg of sample was dispersed on a Glass Bottom Culture Dish (MatTek Corporation, Ashland, MA, 01721) with no. 0 coverglass. The sample was then placed onto the microscope for imaging. An area was selected that had a variety of visibly differentiable constituents. Raman imaging protocols examined an area of 200 μ m by 200 μ m with 10 μ m step sizes. Upon completion of the imaging, the LabSpec software targeted selected peaks for each compound to provide imaging contrast to localize and target the said compound.

While using the inverted microscopes with 10 $\times$ and 40 $\times$ objective lenses, the desired particle(s) were observed and several images were captured, including a bright-field view, a DIC polarizer view; followed by three fluorescence views; all shown in Fig. 1; as well as 40 $\times$ objective bright-field views shown in Fig. S2.† The fluorescence views are produced from three Nikon Fluorescence cubes. The first is a 96310 UV-2E/C C91669 cube which has an excitation wavelength of 340–380 nm and an emission wavelength of 435–485 nm; the second is a 96322 HQ: TR C90554 cube, which has an excitation wavelength of 560/55 nm and an emission wavelength of 645/75 nm; and the last is a 96320 HQ: F C90037 cube, which has an excitation wavelength of 480/40 nm and an emission wavelength of 535/50 nm. To ensure that only the desired particle(s) is being extracted, it was isolated before the

injection and aspiration of the extraction solvent; Fig. 5 illustrates the isolation of a specific particle.

The L200 nanomanipulator (DCG Systems Inc., Fremont, CA, USA) was mounted on the inverted microscope, a schematic of this is shown in Fig. 2, and was equipped with a PM2000B-four channel, 60 psi pressure injector (MicroData Instrument Inc., S. Plainfield, NJ). By the use of a joystick, the probe positioners can be maneuvered in both the course and fine modes, in a three axis motion: *X*, *Y*, and *Z*. While using these modes the nanopositioners have a range of motion in the *X* and *Z* axes of 12 mm and 28 mm in the *Y* axis, with a resolution of 100 nm. An Au/Pd-coated PicoTip EMITTER Econo-12 nanospray capillary tip (New Objective; Woburn, MA) was backfilled with 10 μ L of the extraction solvent, 1 : 1 (v/v) solution of acetonitrile/water and 0.1% of formic acid for sample A, and 70 : 30 (v/v) solution of methanol: water and 1% acetic acid for sample B. The pressure injector uses nitrogen to inject the solvent into the particle with an injection pressure of 0.01 psi and a fill pressure of 4.4 psi. After isolating the desired particle the extraction solvent is injected onto that particle, dissolving it into the solvent. The solvent and the dissolved desired particle are aspirated back into the tip, estimating five minutes for extraction. The capillary tip is then directly moved to the nanospray source in the mass spectrometer (NSI-MS) shown in Fig. 3. This image is a schematic of the nanospray source while the tip is actively spraying into the heated inlet of the mass spectrometer, Fig. 3a. The actual image of the tip as it is aligned and spraying is shown in Fig. 3b, while Fig. 3c shows the coulombic fission event as the sample plus solvent is being sprayed into the heated inlet.

The LCQ DECA XP Plus (Thermo Finnegan; San Jose, Ca) utilizes both ESI and NSI capabilities each with its own set of parameters. Nanospray utilizes a spray voltage of 2.5 kV and an inlet temperature of 200 °C, in the positive mode. As collision induced dissociation (CID) occurs the isolation width is fixed to 2.0 *m/z* while the normalized collision energy percentage is first set at 0 to ensure that the peak we want to fragment is there, and then it is increased to a range of 25 to 30 for fragmentation.

Results and discussion

With the use of a pure melamine standard and known literature values,^{24–26,43–46} the target wavelengths for Raman imaging of the FDA supplied test samples were acquired and used to complete the imaging scan for all compounds: sibutramine, phenolphthalein, and melamine. The Raman spectra and resulting images of the scanned areas are provided in Fig. 4 along with a bright-field comparison image. Fig. 4a–d show the resulting Raman spectra of the respected scanned areas, these spectra are of impure samples. Fig. 4g shows the Raman spectra acquired from a pure melamine standard which displays one major peak of 977 cm^{-1} which appears during the area scan. Fig. 4b shows the Raman image of the sibutramine scanned area, where the pink and yellow regions represent the most intense location of the major peak 1600 cm^{-1} , indicating that sibutramine is present. For comparison, Fig. 4c shows the image of the desired particles of the proposed sibutramine scan using the 10 $\times$ objective with a 100 micrometer scale. Fig. 4e shows the Raman image of the phenolphthalein scanned area, where the blue region represents the most intense location of the major peak 1700 cm^{-1} , indicating that phenolphthalein is present. For comparison, Fig. 4f shows the

image of the desired particles of the proposed phenolphthalein scan using the 10× objective with a 100 micrometer scale. The Raman image of the melamine scanned area is shown in Fig. 4h. The white region represents the most intense location of one of the major peaks of melamine, 668 cm^{-1} , indicating that melamine is present. These peaks are within the range of 378 to 985 cm^{-1} which represent various carbon–nitrogen–carbon bending vibrations and carbon–nitrogen–carbon symmetrical stretching vibrations.²⁵ The bright-field view of the particle using the 40× objective with a 100 micrometer scale is shown in Fig. 4i. The desired particle(s) were selected based on the Raman image and the various views and images, presented in Fig. 1, collected to better characterize each adulterant. The three types of fluorescence shown in Fig. 1 tell us that sibutramine and phenolphthalein are to a certain degree fluorescent whereas melamine is not fluorescent at the previous given wavelengths. Fig. 1c and d show that sibutramine fluoresces and Fig. 1i shows that phenolphthalein fluoresces slightly while melamine does not fluoresce at these wavelengths. As shown in Fig. 1m–o, however, other compounds are visibly fluorescent at these same wavelengths. This allows us to characterize these adulterants as reactive, slightly reactive or unreactive at fluorescence wavelengths of 340–380 nm, 560/55 nm, and 480/40 nm. Subsequently the isolation and extraction of the desired particle(s) occurred and is shown in Fig. 5 and then they were analyzed using the MS. These images show the before, Fig. 5a, and after, Fig. 5b, isolation images of a possible melamine particle using the inverted microscope at a 10× objective. Additionally, a video of this isolation/extraction was recorded; this video demonstrates how the desired particle from sample B was first isolated to a clean new surface and then extracted using the, 70 : 30 (v/v) methanol : water with 1% of acetic acid, extraction solvent.

ESI-MS was used initially to ensure the viability of the extraction solvents for the powders. Fig. 6 shows the LC/MS chromatograms and spectra of sample A. Fig. 6a shows the full and isolated chromatographs of sample A; at approximately 11.2 minutes, the m/z 280 peak elutes and at approximately 12.2 minutes, the m/z 319 peak elutes. Fig. 6b shows the full mass spectrum at time 11.2 minutes with the main MH^+ peak of m/z 280; through the use of MS/MS, fragmentation peaks were produced: m/z 125, 139, and 153, as shown in Fig. 6c. Fig. 6d shows the full mass spectra at time 12.2 minutes with the main MH^+ peak of m/z 319; and again through the use of MS/MS, a fragmentation peak was produced at m/z 225 as shown in Fig. 6e. With further fragmentation (MS^3) of m/z 225, Fig. 6f shows the product peaks of m/z 105, 141, 153, 169, 183, and 197. The two compounds found in sample A are shown in Fig. 7 and 8. Fig. 7a shows the 50–300 m/z region of the full mass spectrum, here we see an MH^+ peak of m/z 280.3. Fig. 7b shows a MS/MS of the m/z 280.3 peak, in addition are the fragmentation peaks including m/z 125.0, 139.1, and 152.9. This fragmentation pattern allowed us to assign the m/z of 280.13 peak to sibutramine.^{4,5,12} Fig. 8a shows the full mass spectrum of the second run completed on sample A, displaying an MH^+ peak of m/z 319.2. MS/MS of m/z 319.2 was performed, resulting in a fragmentation peak of m/z 225.2, which is visible in Fig. 8b. Since no other peaks appeared while performing MS/MS of m/z 319.2 it was decided that MS^3 would be implemented on the m/z 225.2 peak. As a result of this MS^3 , fragmentation peaks of m/z 104.9, 141.0, 153.1, 169.2, and 197.2, emerged as shown in Fig. 8c. These specific fragmentation peaks indicate that the parent peak of m/z 319.0 is phenolphthalein.^{4,12}

Fig. S3† shows the spectra collected from nanospray ionization mass spectrometry of the melamine standard; Fig. S3a† shows the full mass spectrum in which the precursor ion (MH^+) of m/z 127.2 is shown and the most dominant. Through the use of MS/MS the melamine precursor ion was fragmented into product ions resulting in peaks including: m/z 109.2 and 85.0, along with an additional peak of m/z 145.0, as shown in Fig. S3b.† Fig. 9 shows the nanospray spectrum of the extracted particle from the sample; Fig. 9a shows the full mass spectrum, and here we can see an MH^+ (precursor ion) peak of m/z 127.1. The MS/MS spectrum of the precursor ion is shown in Fig. 9b containing fragmentation peaks of m/z 108.9 and 84.9. Based on the comparison to the melamine standard the selected particle from the FDA supplied test sample is identified as melamine. Fig. S4a† shows the chemical structure of melamine. In order to produce the tandem mass spectra, melamine is fragmented to create the multiple product ion peaks that are shown in Fig. 9b. The most abundant peak in the tandem mass spectra is m/z 108.9; this peak is formed when there is a loss of a NH_2 group and a radical hydrogen (from a protonated melamine), as shown in Fig. S4b.† Fig. S4c† shows the loss of two nitrogens, one carbon and two hydrogens, which forms the m/z 84.9 peak. The m/z 145.0 peak is the result of additional water from the solvent binding to the melamine structure, as $M^+ \cdot H_2O$, as shown in Fig. S4d.† In comparison to the melamine standard, shown in Fig. S3,† the melamine structure continuously fragments in the same way leaving an identifiable fragmentation pattern.

Conclusion

Two adulterants, sibutramine and phenolphthalein, found in the “all-natural” weight-loss supplement, sample A, are especially harmful to humans. As of October 2010, the FDA made the announcement to withdraw sibutramine, an obesity/weight loss drug and/or appetite suppressant, from the market due to the risk of heart attack and stroke.⁴⁷ Phenolphthalein is known to many as an indicator for acid–base titrations, but from the 20th century to the late 90s it has also been used in powders and supplements as an over-the-counter laxative.^{6,48} The FDA has not approved phenolphthalein for marketing due to concerns of carcinogenicity.⁴⁹ The melamine adulterant found in the wheat flour product, sample B, has caused much alarm in the past few years. The inclusion of melamine, a nitrogen rich chemical, as an adulterant in human and animal food products to increase the protein content has remained at the forefront of public health concerns. Melamine has current widespread use in plastics, dyes and fertilizers, and textiles, but its inclusion in food products causes renal toxicity and other nephrological detriments.^{40–51} Previous outbreaks have been attributed to mainland China and are still a present concern for the United States and the European Union, influencing the food safety policy.^{52,53} Melamine toxicity in humans, especially in infants, results in the development of kidney stones, especially when ingested with adjuvant chemicals such as cyanuric acid.^{54–57} Furthermore, melamine has been determined to possess carcinogenic properties.¹³ Although the toxicity of melamine and its analogues are well accounted for and a breadth of analytical techniques have been established,⁹ there is still a need for further methods of investigation.

The TE-2000 inverted microscope was adapted and equipped with several other instruments and apparatuses such as the iHR550 Raman Imaging Spectrometer and the nanomanipulator to construct a workstation where a sample could be examined in multiple ways and at one

location to reduce its movement and preparation time to a minimal amount. This workstation allows for a rapid overall examination and testing of a sample. In comparison with LC/MS methods, the direct analyte-probed nanoextraction-coupled nanospray ionization mass spectrometry (DAPNe-NSI-MS) technique reduces the overall experimental time and additionally minimizes the amount of sample needed for analysis. Only trace amounts of each sample were extracted and analyzed, leaving enough to spare for several more extractions for reproducibility. Raman imaging is a rapid scanning method which is also non-destructive which permits the location of a suspected adulterant. As additional Raman spectra are collected from known adulterants the Raman imaging of the sample can be performed rapidly and is noted as a characteristic of the said adulterant. Microscopy offers visual differences including morphology, size, color, and fluorescence. Since this DAPNe technique offers the ability to isolate and extract a single or several particles and immediately analyze them *via* mass spectrometry, this ensures that the results are accurately originating from that previously characterized particle. The Raman imaging spectroscopy coupled to DAPNe-NSI-MS technique makes available several advantages for investigators. Such advantages involve rapid diagnosis/targeting methods, and the selectivity of low concentrations of contaminants provided by Raman imaging; with this method, trace amounts can be pre-detected and image characterized, providing a presumptive identification. Another benefit that occurs due to the ability to extract a single contaminated particle is the increase in the signal to noise ratio from NSI-MS and the confirmation of the adulterant through MS analysis. Finally the DAPNe-NSI-MS method is inherently non-targeted, providing the ability to avoid such problems as dead-time elution of a highly hydrophilic compound in reverse-phase gradients in HPLC-MS. A non-targeted method analysis is ideal for powder/supplement samples that are obtained with little to no background information along with in-accurate ingredient labeling. Overall this combined technique is quite efficient and brings about a prompt presumptive and confirmatory identification.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

TE2000 inverted microscopy images of the samples. Top (a–e): Sample A after sibutramine Raman scan. 10× objective with bullseye marking the targeted particle, 100 micrometer scale. Middle (f–j): Sample A after phenolphthalein Raman scan. 10× objective with bullseye marking the targeted particle, 100 micrometer scale. Bottom (k–o): Sample B after melamine Raman scan. 10× objective with bullseye marking the targeted particle, 100 micrometer scale. (a/f/k) bright-field, (b/g/l) DIC polarizer, (c/h/m) UV-2E/C fluorescence (d/i/n) 96320 HQ: F emits blue and fluoresces green and (e/j/o) 96322 HQ: TR emits green and fluoresces red.

Fig. 2.
Schematic of the nanomanipulator.

Fig. 3.

(a) Schematic of the capillary tip within the nanospray source aligned with the heated inlet of the LCQ DECA XP Plus mass spectrometer and (b) actual image of the tip alignment below the schematic. (c) Enlarged image illustrating coulombic fission as the droplets become ionized and flow into the heated inlet of the mass spectrometer.

Fig. 4.

Using the TE2000 inverted microscope and the iHR550 Raman Imaging Spectrometer with a Synapse detector. Top (a): Raman spectra of the purposed sibutramine, collected from the mixed sample A (b) Raman image after the major peak 1600 cm^{-1} of sibutramine from the literature^{44–46} is scanned, pink/yellow indicates the possible sibutramine contaminant and (c) bright-field, 10 $\times$ objective view of the desired particle(s) of sample A-run 1. Middle (d): Raman spectra of the purposed phenolphthalein, collected from the mixed sample A (e) Raman image after the major peak 1700 cm^{-1} of phenolphthalein from the literature⁴³ is scanned; blue indicates the possible phenolphthalein contaminant and (f) bright-field, 10 $\times$ objective view of the desired particle(s) of sample A-run 2 with a 100 micrometer scale. Bottom (g): Raman spectra of a melamine standard that shows the main peaks of interest, which were used for the scanning (h) the Raman image of the scanned wavelength of 668

cm^{-1} , white indicates the possible melamine contaminant and (i) desired sample B particle in a 40 $\times$ objective bright-field view with a 100 micrometer scale.

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Fig. 5. Images of the sample particle at 10× Objective with a 100 micrometer scale using the TE2000 inverted microscope. (a) Before and (b) after isolation of the desired particle.

Fig. 6.

LC-MS spectra of sample A (a) chromatograms, full (top) and isolated: the m/z 319 peak elution at 12.2 min (middle) and m/z 280 peak elution at 11.2 min (bottom). (b) Full mass spectra at time 11.2 minutes with the main MH^+ peak of m/z 280; (c) MS/MS of m/z 280 showing the fragmentation peaks: m/z 125, 139, and 153; (d) full mass spectra at time 12.2 minutes with the main MH^+ peak of m/z 319; (e) MS/MS of m/z 319.4 showing the fragmentation peak m/z 225; (f) MS/MS/MS of m/z 225 showing the fragmentation peaks: m/z 105, 141, 153, 169, and 197.

Fig. 7. NSI-MS spectra of sample A-run 1 (a) full scan spectra with an MH^+ peak of m/z 280.3; (b) MS/MS spectra of m/z 280.3 showing the parent peak at m/z 279.9 and fragmentation peaks: m/z 125.0, 139.1, and 152.9, identified as sibutramine, the chemical structure is shown in (a).

Fig. 8. NSI-MS spectra of sample A-run 2 (a) full scan spectra with an MH^+ peak of m/z 319.2; (b) MS/MS spectra of m/z 319.2, showing the parent peak at m/z 319.2 and a fragmentation peak m/z 225.2; (c) MS³ spectra of m/z 225.2, showing the isolated fragment peak at m/z 225.2 and fragmentation peaks: m/z 104.9, 141.0, 153.1, 169.2, and 197.2, assigned to phenolphthalein, the chemical structure is shown in (a).

Fig. 9. NSI-MS spectra of the sample B (a) full scan spectra with an MH^+ peak of m/z 127.1; (b) MS/MS spectra of m/z 127.1 (parent peak) with fragmentation peaks: m/z 108.9 and 84.9, along with an additional peak of m/z 145.0; identified as melamine.