Paper spray ionization mass spectrometry for rapid quantification of illegal beverage dyes+ Tianyang Guo,ab Zezhen Zhang,a Karen E. Yannell,a Yiyang Dongb and R. Graham Cooks *a A rapid method is described for quantification of six illegal colorants in beverages, i.e., soft beverages, energy beverages, alcoholic beverages, teas, and fruit juices.Conventional mass spectrometry–based methods for detection of dyes require complex pretreatment process, including drying, extraction, and puri?cation.12,13 These treatments avoid matrix interferences from various mono–, di– and polysaccharides and modi?ed starch, and from compounds with the same molecular weight as the analyte as well as minimizing ionization suppression.14,15 These advantages come with the need for time–consuming chromatographic separation processes.12–14,16 Ambient ionization mass spectrometry17–19 is an alternative way to rapidly ionize samples without or with only minimal pretreatment.Four dyes in aqueous solution were detected by DESI–MS at the ng mL 1 level.27 Dyes with different structures were identi?ed at pg mL 1 level in wool samples by surface acoustic wave nebulization (SAWN) mass spectrometry, although degradation of certain analytes was observed.28 Paper spray (PS) ionization is a representative ESI–related ambient ionization method which allows rapid and direct analysis of an untreated sample from paper or other porous (occasionally non–porous29) media which include a ?ne tip.In the case of foods, food dyes can compensate for color loss, enhance natural colors, or add color to colorless food.1 Synthetic food dyes, synthesized from coal tar or petroleum byproducts, 2 are widely used, owing to their ease of production, low cost, high stability, and good coloring properties.3 During the 1900s, some 80 different food dyes were used in the United States, but over the years, most of them have been taken off the market because of safety concerns.2 Currently, only nine synthetic colorings are allowed for food use by the U.S. Food and Drug Administration (FDA), and these are subject to certi?cation.4 Although maximum use levels for these dyes have been listed by the FDA, their use still requires careful consideration.For example, Fast Green, Orange B and Citrus Red 2 are legal in the US, but they are not permitted in other countries, e.g., China5 and the European Union.6 Some studies showed that synthetic food dyes may have a negative effect on the behavior of children, such as hyperactivity.1,2,7 Furthermore, use of multiple synthetic food colors is also a challenge for food safety, because of potential synergistic effects.8 The addition to food of some industrial dyes, e.g., Sudan dyes, Disperse dyes, Rhodamine B and Malachite Green, is forbidden due to their genotoxic and carcinogenic activity.9 However, some producers still add these illegal non–food colors to the product.10,11 Therefore, a sensitive method to screen both legal and illegal dyes in food is required for food safety.The ESI–related method of desorption electrospray ionization (DESI)20 and APCI–related direct analysis in real time (DART),21 as well as many other ambient ionization techniques, have been applied widely in food safety,19,22 environmental analysis,23 forensics24 and clinical diagnosis.25 As for dyes, several ambient ionization methods have been used for identi?cation or quanti?cation.However, this technique is useful also in the food safety domain, because of the complexity of food matrix and the requirement for trace residue detection.33–37 Some azo dyes at mgmL 1 level have been examined in powdered chili pepper by PS–MS/MS.38 In this paper, we establish a PS–MS/MS method using multiple reaction monitoring (MRM) to quantify illegal dyes in liquid beverages.This technique requires neither elevated temperature nor gas ?ow so it is particularly appropriate for use with portable MS systems.30 In several

application areas, it has been demonstrated to allow aDepartment of Chemistry, Purdue University, West Lafayette, IN 47907, USA bCollege of Life Science and Technology, Beijing University of Chemical Technology, Beijing 100029, China + Electronic supplementary information (ESI) available.Experimental 2.1 Reagents and materials Analytical standards of Sudan I, Sudan II, Malachite Green (as chloride), Rhodamine B (as chloride), Crystal Violet (as chloride) and Methylene Blue (as chloride) were purchased as powders from Sigma–Aldrich (St.PS was performed using a toothless copper clip, obtained from Muller Electric (Akron, OH), and Whatman 1ET–Chr paper, obtained from GE Healthcare Life Sciences (Chicago, IL).Isotopically labeled IS's have performed very well in past ambient ionization mass spectrometry analyses, owing to similar ionization efficiency and similar transport along the paper substrate.31,32 However, the higher cost of isotopically labeled standards makes their use for each individual analyte impractical.LOD and LOQ at ng mL 1 level, accuracy and precision were calculated using matrix–matched calibration curves.In ambient ionization mass spectrometry analysis, IS use is highly desirable.39,40 Commonly, two types of IS are used: structural analogues and stable–isotope labeled forms of analyte.Samples where diluted in methanol and isotopically–labeled internal standards were utilized to obtain linear responses over ranges of two or three orders of magnitude.Two representatives Sudan dyes (Table 1) were selected to assess the performance of rapid determination by paper spray (PS) as described in this article.2.3 Instruments and devices All MS experiments were performed using PS ionization combined with triple quadruple mass spectrometry.Paper spray (PS) ionization combined with tandem mass spectrometry (MS/MS) allowed detection of particular dyes in times of less than 1 min per sample.See DOI: 10.1039/c7ay02241g Cite this: DOI: 10.1039/c7ay02241g Received 18th September 2017 Accepted 25th October 2017 DOI: 10.1039/c7ay02241g rsc.li/methods Anal.The D5 isotopically labeled internal standards Sudan Id5 and Malachite Green–d5 picrate were also purchased from Sigma–Aldrich, and stored at 20 C. Twenty–one colorless and twenty colored beverage samples were purchased from three local supermarkets in West Lafayette, Indiana (Walmart, Meijer, Fresh City Market).View Article Online View Journal quanti?cation of analytes in complex matrices with the use of internal standards.31,32 Most such applications focus on biological samples, such as blood, urine and tissue.HPLC grade methanol, acetic acid, and ammonium acetate were purchased from Sigma–Aldrich (St.2.2 Analytes and internal standards Illegal food dyes of two molecular types were investigated: neutral molecules and salts with complex cations.Internal standards (IS) were used to improve accuracy and precision, as well as to establish the robustness of the method for the various complex matrices.Most neutral molecule dyes produce the protonated molecule [M + H]+ in positive ion mode, while not being ionized in the negative ion mode.The complex cation salt dyes, ionize with relatively high efficiency to give the intact cation [C]+ in the positive mode.High voltage was supplied to the clip and the solvent methanol was pipetted onto the paper triangle.The mass spectrometer used was a TSQ Quantum Access MAX (Thermo Fisher Scienti?c, Waltham, MA).Four Sudan dyes in chili powder were quanti?ed at the mgmL 1 level by DART–MS,26 although DART has proved less successful with those dyes which are salts.Compounds of this neutral molecule class, including Sudan and related dyes, are usually oil–soluble.The examples of this class examined were Malachite Green, Rhodamine B, Crystal Violet and Methylene Blue (Table

1). Introduction Dyes are added to speci?c products to provide attractive visual effects. As a ?nal test, real samples were screened to ensure the usefulness of this method. The IS Sudan I–d5 was chosen for the neutral molecule class and Malachite Green–d5 picrate for the complex salt class (Table 1). The LODs were below 1.5 ng mL 1 and LOQs were below 5 ng mL 1. Matrix effects were evaluated for different samples but had little effect given the use of the dilution method. Methods Analytical Methods PAPER Published on 26 October 2017. Minimal sample preparation was used and internal standards were investigated and optimized. beverages, energy beverages, alcoholic beverages, teas, and fruit juices. Therefore, two IS's were selected to allow detection of multiple analytes. The paper substrate was cut into a small triangle (8 10, mm), and clamped with the copper clip. Quality control experiments gave accuracies that ranged between 80% and 120%. Downloaded by Beijing University of Chemical Technology on 07/11/2017 15:34:28. Louis, MO), and stored at 20 C. Louis, MO). They included so?1.2.