



## NDMA impurity in valsartan and other pharmaceutical products: Analytical methods for the determination of *N*-nitrosamines

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### ABSTRACT

Batch recalls for valsartan containing pharmaceutical products in July 2018 initiated a discussion on possible contaminations with *N*-nitrosodimethylamine (NDMA). It appeared that NDMA was generated during synthesis of the active pharmaceutical ingredient (API) from the solvent dimethylformamide (DMF) and the reagent nitrite. Discussion on NDMA as API impurity is extended to other drugs since then. Already several years before scientific literature reported NDMA as impurity of several other drugs, thus underlining the apparent risk. At present none of the pharmacopoeias tests for NDMA and only very limited publications of methods for its determination in pharmaceuticals are published so far. This review summarizes aspects for the analyses of nitrosamines (NAs) with special focus on NDMA and discusses their potential applicability for drug analyses. The majority of recent publications utilize GC-MS or GC-MS/MS due to its high selectivity and low detection levels. GC-TEA also provides high selectivity for nitrosamines. However, current availability of this combination is very limited. Alternatively, LC-MS/MS is also performed in NA analysis.

An integration of a general test in future pharmacopoeias is suggested due to the toxicological relevance and broader spectrum of possible APIs that may be affected.

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**Abbreviations:** AEM, anion exchange module; APCI, atmospheric pressure chemical ionization; ASE, accelerated solvent extraction; ASTM, American Section of the International Association for Testing Materials; CAR, carboxen; CEP, certificate of suitability; CI, chemical ionization; CLD, chemiluminescence detection; CLND, nitrogen chemiluminescence detector (also abbreviated as NCD); CYP, cytochrome P450; DCM, dichloromethane; DEET, *N,N*-diethyltoluamide; DLE, direct liquid extraction; DLLME, dispersive liquid-liquid microextraction; DMF, dimethylformamide; DVB, divinylbenzene; EDQM, European Directorate for the Quality of Medicines and Healthcare; EI, electron ionization; EPA, United States Environmental Protection Agency; ESI, electrospray ionization; FDA, US Food and Drug Administration; GC, gas chromatography; (HP)TLC, (high performance) thin layer chromatography; (HP)LC, (high performance) liquid chromatography; HRMS, high resolution mass spectrometry; HS, headspace; IARC, International Agency for Research on Cancer; LC, liquid chromatography; LLE, liquid-liquid extraction; LOD, limit of detection; LOQ, lower and upper limit of quantification; MAE, microwave assisted extraction; MS/(MS), (tandem) mass spectrometry; NA, *N*-nitrosamine; NaN<sub>3</sub>, sodium azide; NCD, nitrogen chemiluminescence detector (also abbreviated as CLND); NDEA, *N*-nitrosodiethylamine; NDMA, *N*-nitrosodimethylamine; NEDSA, *N*-1-naphthylethylenediamine sulfanilic acid reagent (in acetic acid); NPD, nitrogen-phosphorous detection; PA, polyacrylate; PCI, positive chemical ionization; PDMS, polydimethylsiloxane; Ph.Eur., European Pharmacopoeia; PR, photoreaction; PTV, programmed temperature vaporizer; RAD54-GFP, reporter system for DNA damage; RP, reversed phase; scCO<sub>2</sub>, supercritical carbon dioxide; SPE, solid phase extraction; SPME, solid phase microextraction; TD, thermal desorption; TEA, thermal energy analyzer; TLC, thin layer chromatography; UHPLC, ultra high performance liquid chromatography; USP, United States Pharmacopeia; UV, ultraviolet detection; WHO, World Health Organization.

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## 1. Introduction

In July 2018 contaminations of valsartan (chemical structure in Fig. 1) containing drugs led to several batch recalls due to their content of N-nitrosodimethylamine (NDMA). It was discovered that changes in the production process of the active pharmaceutical ingredient (API) valsartan led to this as process impurity. First discovered in batches that had been produced by the Chinese company Zhejiang Huahai Pharmaceutical, it turned out in the following weeks that also batches from other manufacturers (e.g. Zhejiang Tianyu Pharmaceutical and Hetero Labs, India) contained NDMA [1]. According to Leclerc [2] this finding was incidental while performing other tests.

The API valsartan is classified as angiotensin II receptor antagonist used in the therapy of hypertension, congestive heart failure, and myocardial infarct. It is listed in different pharmacopoeias together with methods for testing identity, purity and assay. As common, purity testing mainly focusses on expected impurities from synthesis and/or degradation. According to the current monographs for valsartan purity testing focusses on enantiomeric purity, the related compounds ent-valsartan (European Pharmacopeia (Ph.Eur.) impurity A), valsartan benzyl ester (Ph.Eur. impurity B), and desmethyl-valsartan (butyl analogue of valsartan, Ph.Eur. impurity C) as well as residual water in United States Pharmacopeia (USP) and Ph.Eur., and absorbance in USP, while Ph.Eur. requests for sulfated ash as well [3,4].

As mentioned in Ph.Eur. commentary the synthesis of valsartan (Fig. 1) starts from (S)-valin methyl ester and 4'-(bromomethyl)-[1,1'-biphenyl]-2-carbonitrile or 2-cyano-4-formylbiphenyl. As final step the formation of the tetrazol moiety is performed by reaction with azidotributyltin(IV) [5].

In the last years several modified routes for synthesis of valsartan have been published in scientific journals [6–18], representing two different synthetic principles, i.e. formation of the tetrazol ring from a cyano intermediate analogously to the above mentioned route described in Ph.Eur., and biphenyl coupling using an activated tetrazol as educt. In addition, multiple patent applications were submitted. A few of them report the use of sodium nitrite ( $\text{NaNO}_2$ ) in product generation and azide removal [19–21]. Precise description of solvent is missing in these patents. However, the patent of Zhejiang Huahai Pharmaceutical [22] reports tetrazol formation using anhydrous zinc chloride and sodium azide ( $\text{NaN}_3$ ) in aprotic polar solvents, preferably dimethylformamide (DMF), followed by quenching with  $\text{NaNO}_2$ . The limited stability of DMF may have resulted in traces of dimethylamine and subsequent formation of NDMA [23]. Certification of suitability (CEP) for this process seems therefore currently not appropriate.

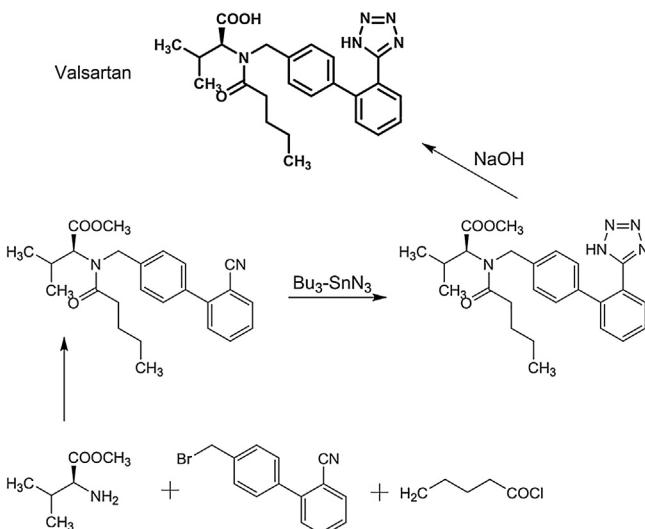
Analysis of valsartan containing drugs in Germany was reported by Abdel-Tawab et al. [24] with concentrations up to 22  $\mu\text{g}$  per tablet found, which corresponds to ~60 mg/kg of API in the tablet. According to recent reports of the US Food and Drug Administration (FDA) concentrations in valsartan API from other manufacturers were lower than those of Zhejiang Huahai Pharmaceutical [1].

In general, N-nitrosamines (NAs) are reaction products of nitrite with amines preferably generated at elevated temperature. Thus, they are mainly detected in food and drinks after processing [25]. Under the regulations of the FDA in beer NDMA is the only regulated nitrosamine with a cut-off value of 5  $\mu\text{g/L}$  [26]. Furthermore, NAs appear as disinfection by-product e.g. in ground and drinking water with maximum concentration levels in the lower ng/L range [27]. Additional contamination of water with NDMA can occur and thus, the World Health Organization (WHO) has set the drinking water guideline values to 100 ng/L [28]. Other organizations such as the U.S. Environmental Protection Agency (EPA) also set limits in their guidelines (EPA screening level of 0.4 ng/L in tap water [29]).

Due to the relevance of NDMA as probable carcinogenic to humans (WHO/IARC group 2 A, EPA group B2, [28,30,31]), and toxic agent [25,32,33], we herein review methods for its detection that have been reported in scientific literature.

## 2. Methods reported for NDMA determination in drug analysis

Only limited numbers of publications report the analysis of NDMA in APIs or pharmaceutical preparations. In the context of the recent batch recalls of valsartan containing pharmaceuticals a publication out of the Central Laboratory of German Pharmacists (Zentrallaboratorium Deutscher Apotheker, Eschborn, Germany) disclosed some analytical method details they applied for the analysis of 16 preparations obtained from German pharmacies [24]. Abdel-Tawab et al. therein report the use of a validated gas chromatography mass spectrometry (GC-MS) based method applying standard addition for proper quantification considering the different matrices of different products. No further details of the method are currently reported. Results found in this investigation showed amounts up to 22  $\mu\text{g}$  per tablet, which represents up to ~30 mg/kg



**Fig. 1.** Chemical structure of valsartan and synthesis pathway according to Ph.Eur., commentary.

in the preparation. Very recently, the FDA issued a GC-MS method with headspace (HS) injection and electron ionization (EI) for detection of NDMA in valsartan drug substance with a reported limit of quantification (LOQ) of 0.3 mg/kg [34]. No excipients appeared to be considered in this method yet. The API is dissolved in DMSO directly in the HS vial and equilibration is carried out for 15 min. Within the review period of this article a few new methods have been reported that are optimized for the analysis of pharmaceutical preparations [35]. Analogously to the FDA method a HS-GC-MS was used for NDMA screening and quantification in API and powdered tablets that are dissolved in DMSO. In screening the limit of detection (LOD) was 10 mg/kg. The application of a more sensitive second method starting with a higher amount of test sample is recommended if no NDMA was detected in the initial method. This method allows for detection at LOD = 0.04 mg/kg [36]. Another method utilizes HPLC-APCI-MS/MS after methanolic extraction from the homogenized samples and dilution with water. LOQs of 0.2 mg/kg for NDMA and 0.04 mg/kg for N-nitrosodiethylamine (NDEA) were achieved [37]. Finally, a method utilizing HPLC-UV was reported with LOD = 0.1 mg/kg [38]. For sample preparation this method also includes methanolic extraction of the homogenized tablet(s), dilution with water, and filtration of the injection solution. Quantification is performed by standard addition methodology.

Already almost 30 years earlier, Castegnaro et al. [39] reported the analysis of pharmaceutical preparations containing aminopyramine, disulfiram or oxytetracycline using GC in combination with thermal energy analyzer (TEA) for detection of volatile N-nitrosamines. They used a packed GC column in isothermal mode. Sample preparation used 1 g of powdered drug, 6 g of glucose, 1 g of ascorbic acid, 20 mL of paraffin oil, and 10 mL of water plus 0.5 mL of sulfuric acid (5 N). Vacuum distillation was performed with trapping of NDMA at liquid nitrogen cooling. Further purification included liquid-liquid extraction (LLE) using aqueous NaOH (0.2 N) and dichloromethane (DCM) and subsequent solid phase extraction (SPE) on alumina. Out of the 46 different drug formulations containing aminopyramine, or oxytetracycline only one did not contain any detectable NDMA. Reported levels of the other preparations were in the range of 1 µg/kg up to 90 mg/kg of NDMA averaged from one full box of preparation. Individual concentrations in the tablets were reported to show large variances. All preparations containing disulfiram contained N-NDEA at concentrations of 94–980 µg/kg.

A similar method was reported by Eisenbrand et al. [40] for the analysis of aminopyrine drugs. They found NDMA in all of the 68 tested preparations at concentrations of 1–371 µg/kg. Due to strong variations within batches, they hypothesized the high reactivity of API towards nitrogen oxides as reason. Furthermore, Taylor et al. [41] reported low levels of NDMA and NDEA in antihistaminic drugs (containing diphenhydramine, doxylamine, and methapyrilene).

Moreover, Dawson and Lawrence [42] found 21 out of 34 preparations to contain NDMA at levels up to 12 µg/kg. The LOD and LOQ were determined at LOD = 0.1 µg/kg, LOQ = 0.3 µg/kg. Again, GC-TEA was used with a packed column at isothermal conditions. For sample preparation they used extraction of NDMA from both, liquid or solid, preparations by aqueous sulfamic acid solution. DCM may be used as solubilizer. Subsequent distillation, extraction of the distillate, and further concentration resulted in the final injection solution. The APIs involved were amitriptyline, chloramphenicol, chlorpromazine, dimenhydrinate, erythromycin, imipramine, promazine, propoxyphene, trimipramine, and tetracycline. No increasing NDMA concentrations were found in preparations after the expiry date. Further five preparations were found to contain NDMA as well, however contaminations and not reaction products from the API are claimed as reason in these cases. Some of these preparations were stored in vials sealed with rubber septa that were found to contain NDMA. Thus, they hypothesized this as possible source.

In contrast, Krull et al. [43] found none of the 73 products tested within their study to contain NDMA, however three interfering signals from drug ingredients themselves. They utilized GC-TEA as well as high performance liquid chromatography (HPLC) hyphenated with TEA and report detection limits of 1 µg/kg. Powdered samples were extracted with acetone and the supernatant injected directly.

Further methods for API analyses are reported in the context of investigations on NDMA formation potential rather than drug testing for NDMA. As one example Shen and Andrews [44] reported the analysis of nine nitrosamines (NAs) formed after treatment of 20 pharmaceuticals and personal care products with disinfectant chloramines. After incubation of the drug with chloramine in phosphate buffer NDMA and NDEA were extracted from the solution using divinylbenzene (DVB) styrol copolymer beads (dispersive SPE) and eluted with DCM. Analyses were performed by GC-MS using chemical ionization (CI) with methanol as reagent gas. Separation was achieved on a capillary column and temperature gradient. Large volume injection was performed using a programmed temperature vaporizer (PTV) inlet with a Carbofrit liner. Amitriptyline, azithromycin, carbinoxamine, chlorpheniramine, clarithromycin, diltiazem, doxylamine, erythromycin, escitalorpram, metformin, nizatidine, ranitidine, roxithromycin, sumatriptan, tetracycline, tramadol, and venlafaxine were reported to form NDMA at ~0.3% or more under the conditions applied. Similarly, Leavey-Roback et al. [45] found NDMA at conversion rates >0.04% after incubation of chlortetracycline, clarithromycin, meropenem, minocycline, oleandomycin, oxytetracycline, quinupristin, spiramycin, and tetracycline. They used amborsorb for SPE and GC with tandem mass spectrometry (MS/MS) using CI as reported by Cheng et al. [46].

Alternatively, Wang et al. [47] reported the use of LC-MS/MS with electrospray ionization (ESI) for the detection of NDMA after incubation of drugs with different oxidants. Sample preparation was performed by SPE on activated charcoal after oxidation of the drug in phosphate buffer. Separation was carried out on a RP-18 column using acetonitrile and aqueous formic acid (0.1%). They reported NDMA after incubation of carbinoxamine, doxylamine, nizatidine, and ranitidine. Applying the same methodology, Lv et al. [48] reported amitriptyline, chlorpromazine, chlorprothixene, citalopram, clomipramine, doxepin, and venlafaxine as precursors of NDMA.

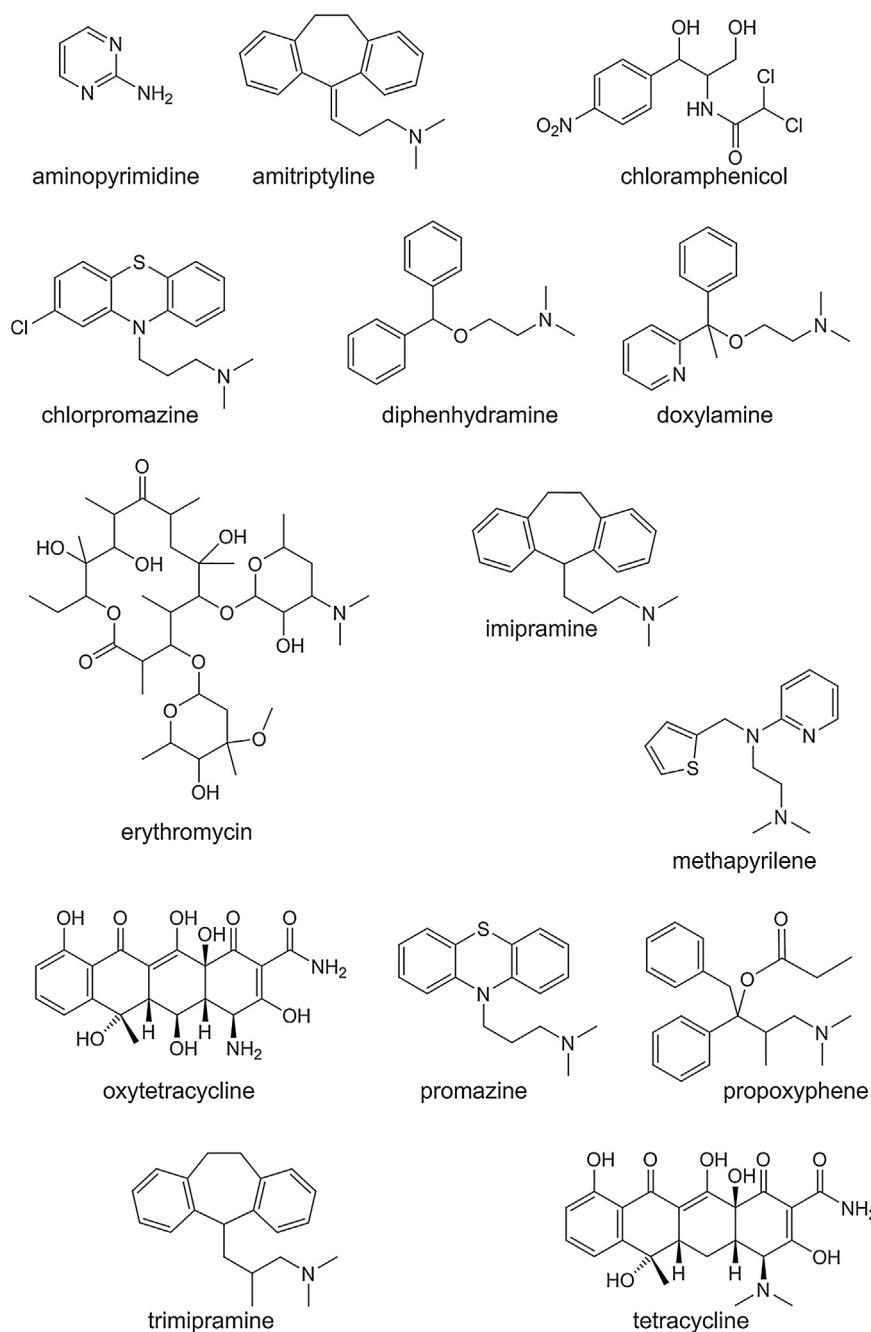
The structure formulae of all APIs reported to contain NDMA as mentioned above are shown in Fig. 2, while chemical structures of additional NDMA precursors are shown in Fig. 3.

### 3. General analytical methods for the determination of NDMA

The following section gives an overview on analytical methods that are reported for NDMA determination. Besides a brief introduction of the respective technique method performance characteristics are briefly reported. A direct correlation of the performance characteristics, especially achieved LOD or LOQ with the analytical technique used for determination may be hampered by underlying effects. Apart from the detection technique, method performance characteristics may be considerably influenced by type of matrix, utilized way of sample preparation, and/or different instruments even if based on the same detection principle.

#### 3.1. Gas chromatographic methods

More generally, most of the reported methods for NDMA analyses in scientific literature utilize GC separation. Moderate to high polarity stationary phases are used for separation with carbowax

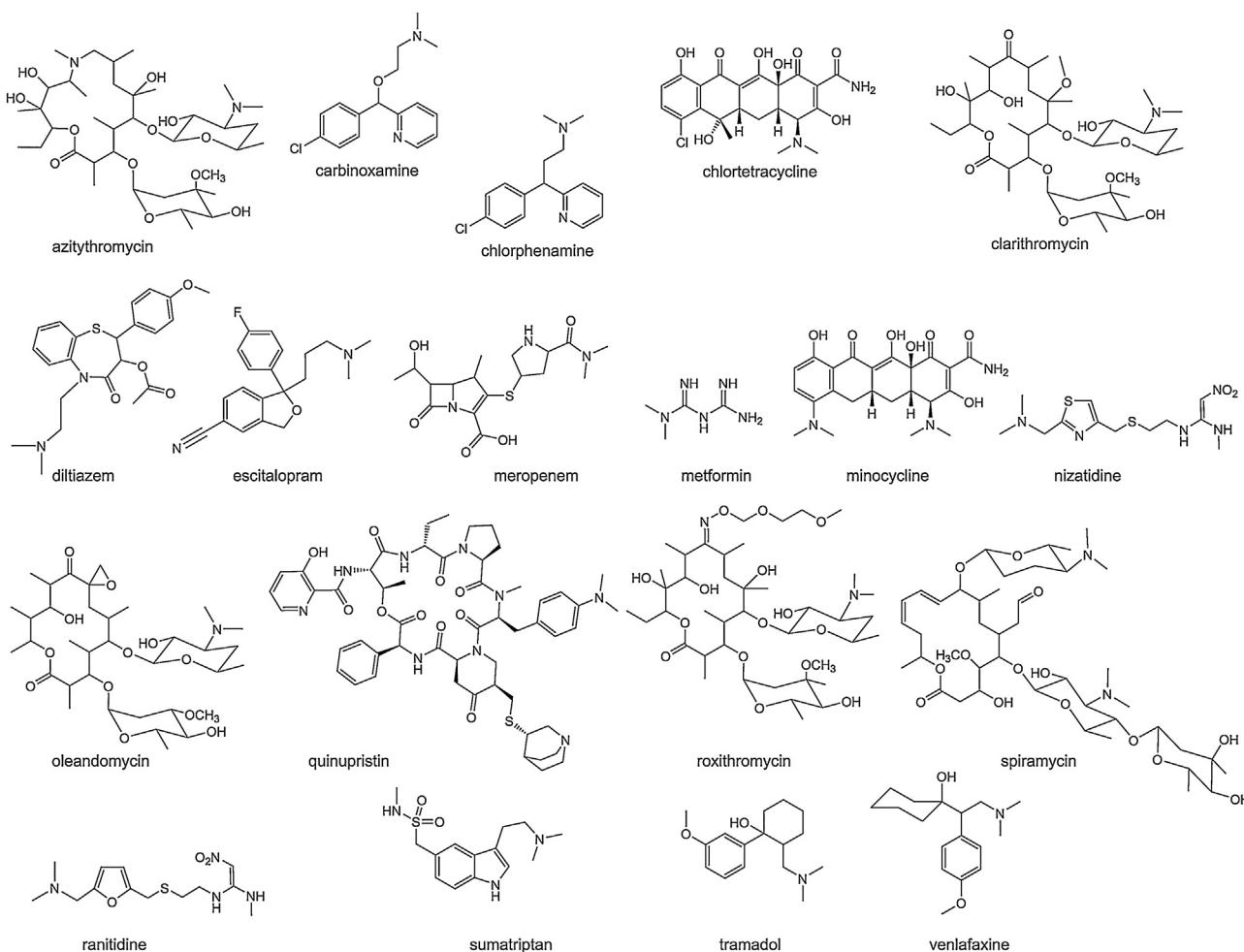


**Fig. 2.** Chemical structures of APIs reported in literature to contain NDMA.

or analogous material being most popular [49–52]. This material was also used in the recently published method of the FDA [34]. In contrast, the United States Environmental Protection Agency (EPA) method for N-nitrosamines (NAs) in drinking water recommends a polyphenylmethylsilicone column [53]. In this column type the surface is modified by polyethyleneglycol residues to allow for additional dipol-dipol and hydrogen bonding of analyte and stationary phase. This may lead to improved separation of polar analytes. Chromatograms of different NAs on HP-5 and DB-Wax columns are shown in Fig. 4.

Very few manuscripts report the detection of NAs by GC-FID [54–56] or GC-ECD after oxidation to their corresponding nitramines [57–59] or heptafluorobutyryl derivatives of corresponding amines liberated from the NAs [60]. The latter is reported to even detect pg amounts [58], however oxidation may lack repro-

ducibility [61]. More frequently the detection of NAs is performed by hyphenation of GC with nitrogen-phosphorous detection (NPD) [32,51,54,62–66] or nitrogen chemiluminescence detector (NCD or CLND), which is also named thermal energy analyzer (TEA). Reports of the use of chemiluminescence detection (CLD) in the special nitrosamine mode already date back to 1974 [67]. Using this detector NAs are pyrolyzed. The liberated NO radicals are subsequently oxidized and detected as NO<sub>2</sub> after excitation by light emission in near infrared [67–69]. Its use for NDMA detection out of different matrices is reported in several manuscripts (e.g. [65,66,69–84]). Very high selectivity also in complex matrices such as food as well as low LOQ can be achieved by GC-NCD. The combination of the separation of analytes by GC and the selective detection of compounds with labile nitrosyl group as structural element both contribute to the high selectivity of this system [68].



**Fig. 3.** Chemical structures of additional NDMA precursors, observed from disinfection by-product testing.

Hyphenation of GC separation with MS detection [34,36,44,50,51,85–98] allowed to achieve LODs even suitable for the analyses of drinking water. The current reference method for the analysis of NAs in drinking water requires the use of GC-MS/MS due to its very low detection limits together with positive chemical ionization (PCI) using methanol or acetonitrile as reagent gas [53,99]. Further methods are reported utilizing PCI-MS/MS with methanol [46,100–104], or less frequently ammonia [52,54,98,105,106], or methane [107,108]), EI-MS/MS [90,109–117], or high resolution mass spectrometry (HRMS) [118,119]. It was found that CI yields better specificity than does EI and often provides higher sensitivity in NA analysis [98,104,107]. Chen et al. [114] compared GC-MS with GC-MS/MS for environmental water matrices and found instrument detection limits in GC-MS/MS 100-times lower (0.5 µg/L in GC-MS/MS and 50 µg/L in GC-MS).

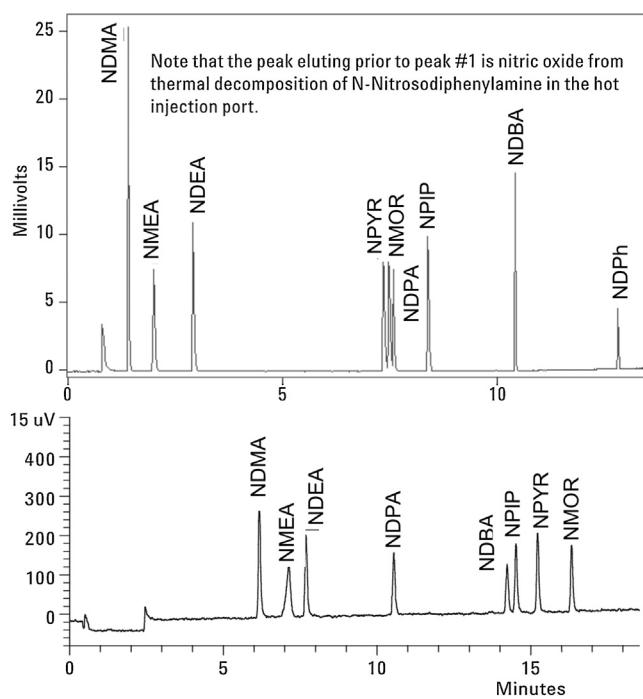
Especially for NDMA analyses are mainly carried out using d<sub>6</sub>-NDMA [53,89,100,105,107,108,120] as internal standard in GC-MS. For GC-NCD N-nitrosodiiso- or -n-propylamine [70,71,74] were reported as internal standards as this detector requires chromatographic separation of analyte and internal standard. Alternatively, N-nitrosodi-n-butylamine was used as internal standard for GC-NCD analyses [72,75,78].

A comparison of the method performance depending on different detectors was reported in 2006 by Grebel et al. [66]. They found method detection limits for NDMA in GC-NCD at 57 ng/L, while GC-Cl-MS using ammonia as reagent gas yielded ~50% LOD

(i.e. 30 ng/L) and GC-NPD considerably higher (890 ng/L) using the standard deviation method for data evaluation. Jurado-Sanchez et al. [54] reported LOD and LOQ in GC-NPD around seven times higher compared to GC-Cl-MS using ammonia. GC-FID was found less sensitive by more than one order of magnitude therein. Precision was reported very similar for all three detectors in their set-up. Only slightly lower sensitivity in GC-NPD in comparison to GC-NCD was reported by Grebel and Mel Suffet in 2007 (instrument detection limit ~50% higher). In terms of accuracy the comparison of water sample analyses utilizing GC-NPD, GC-NCD as well as GC-Cl-MS(/MS) resulted in statistically insignificantly differing results [65].

For injection of the samples onto the GC system different sample inlets were utilized: classical split/splitless inlet [65,96,106,121], programmed temperature or cold injector [44,50], as well as thermal desorption (TD) or headspace (HS) inlet [34,36,83,122,123]. The use of a HS sample inlet often in combination with solid phase microextraction (SPME) is reported for various liquid and solid matrices [66,79,86,92,101–103,115,124–129]. As closely related technique headspace-single drop microextraction is reported for the analysis of N-nitrosodipropylamines using a single drop of toluene instead of the SPME fiber [130].

Headspace injection demonstrated to be advantageous compared to split/splitless injector, programmed temperature and cold injector due to its increased specificity and matrix reduction [92]. Especially in the analysis of trace amounts of volatile NAs such as NDMA HS-SPME allowed to reach very low LOD and LOQ, e.g.



**Fig. 4.** Gas chromatographic separation (GC-TEA) of different NAs on HP-5 column and split injection at 200 °C ( $c = 2 \mu\text{g/mL}$ , upper) and DB-Wax column and on-column injection at injector temperature of 53 °C ( $c = 100 \text{ ng/mL}$ , lower), courtesy of Agilent Technologies, Inc. [132].

LOD(water) = 0.5 ng/mL and LOD(beer) = 12 ng/mL when combined with GC-CI-MS [128]. However, careful method development in HS-SPME is necessary to obtain robust determinations [92]. Equilibrium and extraction temperature and time, as well as ionic strength of the solution were found to affect peak areas [66,92,126]. Andrade et al. recommend factorial design for HS-SPME optimization [79].

As SPME fiber materials polyacrylate (PA) [79,125], polydimethylsiloxane (PDMS) [125] as well as mixed mode divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS) [101], carbowax-divinylbenzene (CW-DVB) [66], PDMS-DVB [79,92,126], and CAR-PDMS [66,86,101,115,124,125] are reported for NA analysis. For NDMA PDMS-DVB fibers resulted in better recoveries compared to PA [79,125]. DVB-CAR-PDMS was found superior to CAR-PDMS [101] and to PDMS-DVB, CW-PDMS, PDMS and PA [66,125]. Carbon based extraction disks were reported for drinking and groundwater analyses as well [131]. A thermal desorption inlet system is used therein.

A very recent publication shows a modified system where HS-SPME was performed under vacuum assistance [124]. In this case the headspace vial is evacuated prior to sample injection. This procedure was reported to result in ten times higher peak areas for NAs at extraction times of 30 min at 50 °C. However the authors mention that automation is not (yet) possible, which limits its application.

Alternatively trapping of NAs on Tenax-TA liners and thermal desorption (TD) may be performed [83,122]. Invaco et al. [123] reported TD using Thermosorb/N as superior material due to inhibition of nitrosamine formation on the adsorbent as artefact.

Furthermore, cold injection is advantageous to hot injection due to the limited stability of NAs that are susceptible of thermal decomposition to nitric oxide (NO) in the injector [118]. This is especially seen in labile NAs such as *N*-nitrosodiphenylamine (Fig. 4, [132]). Due to the low boiling point of NDMA ( $T_B = 154^\circ\text{C}$  [133]) hot injection may also lead to peak broadening and therefore reduced sensitivity.

### 3.2. Liquid chromatography based methods

Alternatively, high performance liquid chromatography (HPLC) on reversed phase columns (mainly RP, C-8 or C-18) is performed for NDMA determination. Detection by ultraviolet (UV) was reported by Al-Kaseem et al. and Li et al. [134,135] using diode array detection (DAD) at a wavelength of 230–233 nm, respectively. The recently published method for the detection of NDMA in valsartan API and tablets uses a detection wave length of 228 nm [38].

LC with tandem mass spectrometry (LC-MS/MS) [37,48,112,115,136–155], LC-HRMS [156,157], or ion mobility HRMS [158] were used in combination with HPLC separations. Electrospray ionization (ESI) [112,115,136–140,142,143,156,157] or atmospheric pressure chemical ionization (APCI) [37,138,141–147] are used therein. HPLC-TEA is also possible [80,159–161], however, inferior to GC-TEA especially in the analysis of volatile NAs, such as NDMA. Nevertheless, a HPLC based method advantageously detects both thermally stable and unstable NAs. A lack of reproducibility was reported by Loepky if aqueous mobile phases are used [162].

Reproducible analyses of NAs were reported by liquid chromatography using post-column photolysis and chemiluminescence (LC-PR-CLD), which is also marketed as Nitrolite detector [163–167]. The use of tris(bipyridyl)ruthenium(III) in CLD resulted in detection limits of 300 ng/L [163]. Comparison of reversed osmosis LC-PR-CLD was reported similar to SPE-GC-MS/MS in terms of accuracy and LOD [168]. Alternative to the above mentioned HPLC on RP columns, *N*-nitrosodiethylamine (NDEA) was determined using ion chromatography [169]. In this study, photolysis of the NA and subsequent chromatography on an anion exchange column was performed utilizing a conductivity detector.

HPLC combined with fluorescence detection after pre-column derivatization of secondary amines liberated from the NAs by cleavage with HBr in acetic acid was reported [170–172]. Derivatization is performed by dansyl chloride (5-(dimethylamino)naphthalene-1-sulfonyl chloride) [170,171], 2-(11H-benzo[a]carbazol-11-yl)ethyl carbonochloridate [172], or 1-fluoro-2,4-dinitrobenzene [173]. Similarly, (high performance) thin layer chromatography ((HP)TLC) separation may be combined with fluorescence detection [61,174]. Direct TLC of intact NAs is reported by Sen and Dalpe [175]. Detection was possible after spraying with Griess (sulphanilamide and N-(1-naphthyl) ethylenediamine in concentrated hydrochloric acid) or NEDSA reagent (sulfanilic acid and N-1-naphthylethylenediamine hydrochloride in acetic acid) [175–177].

### 3.3. Non-chromatographic methods

Several methods for the analysis of NAs were reported that do not apply chromatographic separation. They are generally considered as less selective and often used for screening or for determination of the sum of NAs.

Polarography, initially reported for successful NA determination, later turned out to lack selectivity and its use seems to be discontinued in NA analyses [61].

Ceto et al. reported the use of a direct electrochemical detection with molecular imprinted polymers as membrane of an impedimetric sensor for water analyses [178]. A LOD of 0.85 μg/L was achieved applying this sensor.

Total NA concentrations in water were obtained using UV-photolysis and chemiluminescence detection [179]. Spectrophotometry after photolysis and Griess reaction of liberated  $\text{NO}_2^-$  with sulphanilamide and N-(1-naphthyl) ethylenediamine in concentrated hydrochloric acid was used in food industries for lower cost [180]. However, Li et al. reported that concentrations of the generated  $\text{NO}_2^-$  and  $\text{NO}_3^-$  ions vary with photolysis conditions in NDEA analysis resulting in inconsistent measurement because it

is based on the amount of photoproduced  $\text{NO}_2^-$  [169]. Furthermore, the method is only suitable for considerably high concentrations equivalent to high  $\mu\text{g/mL}$  concentrations of NAs.

Another colorimetric assay is based on the Eisenbrand Preussmann reaction [69,181]. After cleavage of the nitrosamine using HBr in acetic acid the resulting nitrite is detected by colorimetry. Nitrite was also reported to be liberated from NAs by photolysis and combined with a colorimetric assay [182]. Final detection is based on the formation of a colored product from the liberated nitrite. It may form a violet-purple complex upon reaction with  $\text{PdCl}_2$  and diphenylamine (Preussmann reagent), however, false positive results have been reported [61]. Alternatively, the liberated nitrite was subjected to Griess reaction (with sulfanilamide and N-(1-naphthyl)ethylenediamine in concentrated hydrochloric acid) [54]. Liebermann nitroso reaction (reaction with phenol to p-nitrosophenol, reaction with a second phenol to a blue quinon-imine) [183] or reaction with NEDSA reagent [182]. The latter is performed in an automated design as reported by Fan and Tannenbaum [182]. In general colorimetric assays were reported to have relatively high limits of detection with reports of 0.1  $\mu\text{g}$  of NDMA [54] or even mg amounts necessary for detection [183].

In 2005, Walsh et al. [184] reported the use of a yeast based biosensor system for the detection of carcinogens and procarcinogens. It is based on genetically modified yeast strains that express a reporter system for DNA damage (RAD54-GFP). For activation of procarcinogens modified strains with additional cytochrome P450 (CYP) enzymes were also introduced. It was tested for the detection of NDMA resulting in a positive signal at a concentration of 1.6 mg/L. Bui et al. [185] used a similar system and found a positive signal at a concentration of 3 mg/L (40 mM). However, it needs to be noted that the use of these assays may be hampered by considerably high concentrations of the API in pharmaceutical product testing. For example in the recent valsartan analyses the API-NDMA ratio was ~15,000 or even higher. Thus, intensive purification may be required. Furthermore, very high amounts of pharmaceutical product would be required to reach the necessary amount of NDMA, making the use of this system impossible for drug testing.

#### 4. Sample preparation

As mentioned in chapter 2 only few methods are available from literature for the analysis of NDMA in drugs or pharmaceutical products. They most frequently report the use of distillation, LLE, and/or SPE for sample preparation. However, different pitfalls of complex sample preparation steps are reported in the analysis of NAs in different matrices.

Dichloromethane (DCM, methylene chloride), the most prominent extraction solvent for NAs, may contain NDMA and even distilled water was found to result in an interfering peak in NA analysis that could be eliminated if washed with DCM prior to use [42]. Furthermore, DCM and chloroform have been found to contain N-nitrosomorpholine in concentrations of 2–376  $\mu\text{g/kg}$  (27–40% of the samples) [186].

Special precaution during analysis of NAs, especially NDMA, is necessary since pH dependent instability of NDMA [66] and photolysis [138] are reported, which may cause underreporting of NA concentration. In contrast, overestimation of NA concentrations may result from nitrosation of amines present in the sample. This NA formation was especially reported as disinfection by-product in water analyses but also a combination of secondary amines or amino acids with nitrite in the sample may result in additional NA formation during sample preparation [187].

#### 4.1. Solid phase extraction (SPE)

SPE for NDMA concentration in combination with isotope dilution is mainly used in the field of water analysis. Especially relevant for ultra-trace analysis of NAs in ground and drinking water very high enrichment factors may be achieved. Thus, the EPA method 521 implies the use of SPE for concentration of water samples prior to GC-Cl-MS/MS [53,99]. Other methods also report the use of SPE for NA pre-concentration and purification in combination with GC-MS [44,50,85,98], GC-MS/MS [52,90,100,104,105,114,116,117,188,189], and GC-NCD [82]. The latter uses two-step SPE cleanup for meat products prior to GCxGC-NCD.

In combination with HPLC based methods SPE appears the method of choice for NA extraction and various methods are reported for LC-MS/MS [48,136,140–142,144–155] and LC-HRMS [156] as well as HPLC hyphenation with post-column UV photolysis and Griess reaction [190]. Automation of SPE is possible especially in combination with HPLC. As one example, Hu et al. were able to analyze nine different NAs in human urine using a combination of one manual and an online SPE [147]. Furthermore, Breider et al. developed a method for determination of total nitrosamine content utilizing UV chemiluminescence of nitric oxide and tested their method with SPE pre-concentrated water samples [179].

The most popular sorbent employed in the SPE of NAs is activated carbon, as proposed by the EPA method 521 for water analysis (e.g. [48,55,85,90,104,107,114,148–150,153,154,156]), which was also reported for urine analysis [50,144,147]. Oasis HLB phases were used for water [146,157,179,190] or cosmetic product analysis [117]. Extrelut [82,91], Florisil [82,91,105] and Ambersorb 572 [45,170], LiChrolut® EN [98], Enviro-UCT [146], Sep-Pak Plus® AC-2 [142], and Bakerbond Carbon [157,190] phases are further examples of NA compatible SPEs.

Alternatively to classical SPE the extraction of NDMA and eight further nitrosamines from salted fish or soy sauce was realized by dispersive solid phase extraction using so-called QuEChERS. During extraction with QuEChERS the analytes are adsorbed on the particles but generally no cartridges are used [110–113,120]. Similarly, NDMA extraction was performed using dispersive micro solid-phase extraction with carbon molecular sieves [191,192].

#### 4.2. Liquid-liquid extraction (LLE)

Dichloromethane (DCM, methylene chloride) is the most prominent extraction solvent for NDMA and other NAs. Successful LLE of NDMA was reported for water [89], beer and cigarette smoke [171], artificial saliva (migrated from rubber teats and soothers or baby bottle teats and rubber kitchen tools, respectively) [193,194], the migration from condoms [195], or in human urine utilizing an automated approach [196,197]. Alternatively, mixtures of DCM or acetonitrile were used for LLE. A mixture of DCM and isopropyl alcohol and subsequent analysis via GC-EI-MS/MS was reported for gastric juice samples [198]. Herrmann et al. [143] extracted different nitrosamines with acetonitrile prior to LC-MS/MS for NDMA detection. Multi-step LLE utilizing DCM, ethanol, methanol, and aqueous sodium chloride was applied for NDMA analysis utilizing HPLC-UV [135] and further combined with SPE.

Dispersive liquid-liquid microextraction (DLLME) was reported for use in combination with HPLC and fluorescence detection [172] and a combination of DLLME and prior microwave assisted extraction followed by GC-MS [94,96,97] analysis.

In general, LLE is considered time-consuming and labor intensive, may require large volumes of sample and solvents, and suffers high risk of contamination and analyte losses [78,89].

#### 4.3. Direct liquid extraction (DLE)

Analysis of thirteen nitrosamines in cosmetic products via GC-MS was accomplished after direct liquid extraction (DLE) with water and acetonitrile [95]. A pressurized liquid extraction of house dust with ethyl acetate to detect nitrosamines from third hand tobacco smoke was performed prior to subsequent analysis either by GC-MS or GCxGC-NCD [199,200]. Hot water pressurized liquid extraction was utilized for the extraction of nitrosamines from sewage sludge prior to HS-SPME and subsequent GC-MS/MS detection with positive chemical ionization using methanol [102]. Direct liquid extraction of nine nitrosamines from atmospheric particulates with DCM and ultrasonication without further concentration of the sample volume and consequent analysis by GC-MS/MS was reported by Hong et al. [109]. A similar extraction protocol, but following analysis of NDMA by LC-ESI-MS/MS in biosolids (sewage sludge or freshwater sediments near wastewater treatment plants) is also reported [201,202].

#### 4.4. Distillation

Steam or vacuum distillation of volatile NAs commonly followed by LLE as sample preparation step was often used in food analysis [175,203–211]. Analysis of NDMA in food products and air by a colorimetric approach was realized after several extraction steps and distillation [212]. Castegnaro et al. [39] reported the observation that for pharmaceutical products steam distillation from water quite often led to foam generation. Thus, they used a modified procedure from mineral oil with added water.

#### 4.5. Other sample preparation methods

Soxhlet extraction of NAs is reported, among others, for soil, sewage sludge, and sediments, or even mandatory in testing methods regarding NA content in rubber teats and soothers (American Section of the International Association for Testing Materials (ASTM) F1313-90 [213] or EN 12868 [214]). As Soxhlet extraction solvent DCM is often used. The extraction of NDMA from sludge as pretreatment prior to SPE and subsequent analysis via GC-MS is reported by Chen et al. [85]. A comparison of extraction efficiency between Soxhlet extraction, microwave assisted, and ultrasonic assisted extraction followed by continuous SPE and GC-MS analysis revealed best results for microwave assisted extraction (MAE) [215].

Alternatively, pressurized hot water extraction or accelerated solvent extraction (ASE) was also successfully applied in the analysis of NAs in sewage sludge [102]. Supercritical fluid extraction (SFE), i.e. extraction using supercritical carbon dioxide ( $\text{scCO}_2$ ), of NAs from rubber materials and GC-NPD detection showed only 8% recovery for NDMA [63]. Fiddler and Pensabene on the other hand compared SFE with SPE, mineral oil distillation and low-temperature vacuum distillation regarding NA extraction from fried bacon resulting in best recoveries for SFE (detection GC-TEA) [216]. The combination of SFE-SPE with subsequent GC-TEA detection reached a LOD of 1  $\mu\text{g}/\text{kg}$  for NDMA [71].

As reported by Kodamatani et al. no prior sample concentration was necessary using LC-PR-CLD (chapter 3.2). Using this indirect method for the analysis of NDMA in water samples LOD = 1.5 ng/L could be achieved anyway [164]. Introducing an anion exchange module (AEM) after the HPLC column and prior to the photoreactor further decreased the method detection limit to LOD = 0.09 ng/L for NDMA [217].

Similarly no sample preparation was necessary according to Ceto et al. who used a direct electrochemical detection with molecular imprinted polymers (LOD 0.85  $\mu\text{g}/\text{L}$ ) [178].

#### 5. Conclusions and future perspectives

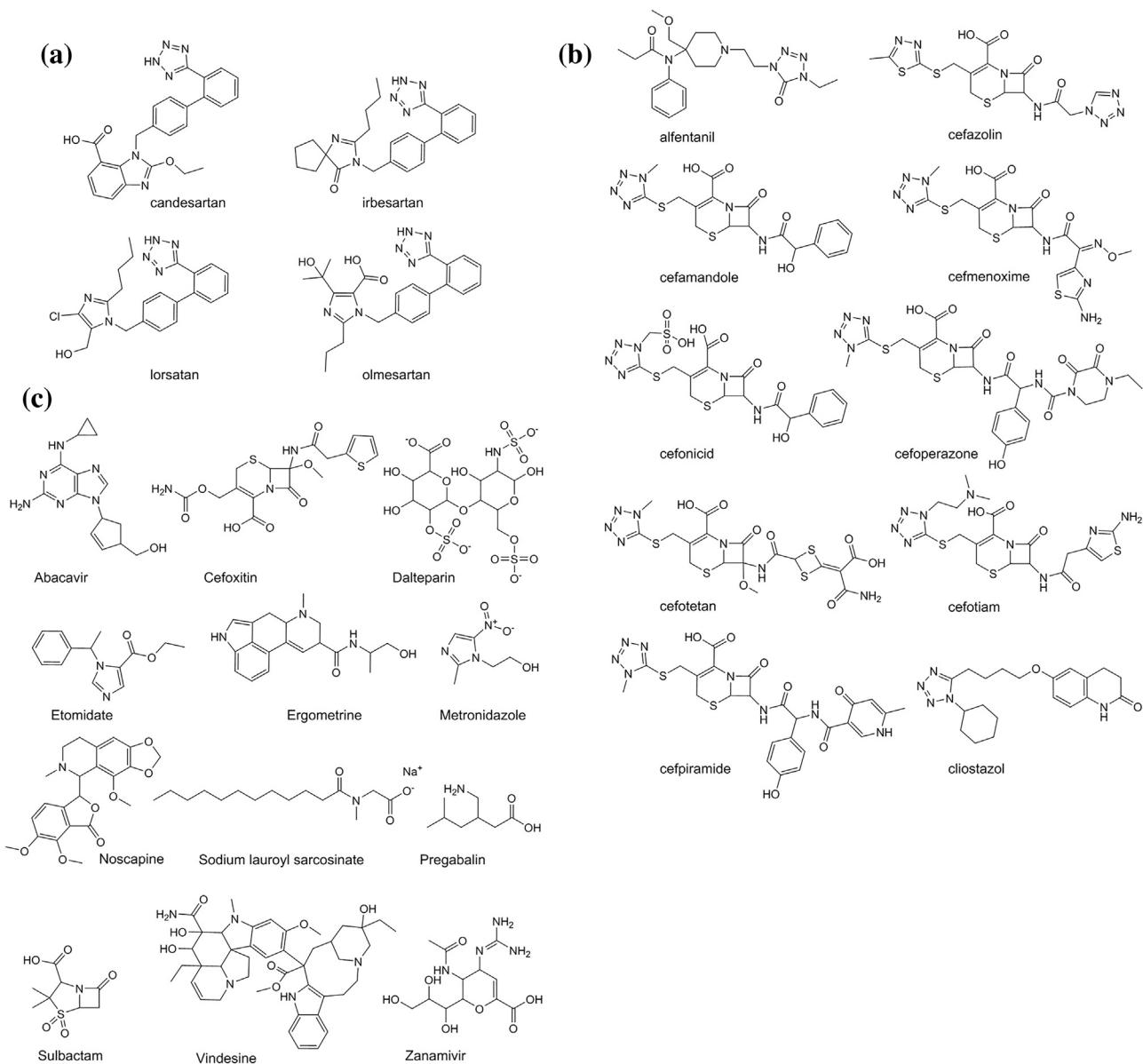
Several methods for the detection of NDMA are already available from literature, however most frequently focusing on matrices different from drugs. In recent times, most of these methods are based on GC-MS(/MS). Sample preparation may be adapted to the needs of drug analyses. Straightforward sample preparation was recently reported by the FDA for valsartan drug substances [34] or valsartan API and preparations by the OMCL laboratory Galway [36]. Therein GC-EI-MS using headspace from a solution of valsartan in DMSO resulted in an appropriate sensitivity of the method for the recent valsartan contaminations (FDA: LOQ = 0.3 mg/kg, Galway: LOD = 0.04 mg/kg). This method offers fast and easy analysis but sample preparation may need extension depending on the type of excipients if drug preparations are considered as well. The recently published HPLC based methods applied methanolic extraction from the powdered tablet matrix. A reporting limit of 0.3 mg/kg is set in the HPLC-UV based method, while LOQs of 0.2 mg/kg for NDMA and 0.04 mg/kg for NDEA are reported utilizing HPLC-APCI-MS/MS [37]. Earlier reported methods (e.g. Dawson and Lawrence [42]) reported considerably lower LOQs for pharmaceutical products using GC-TEA, however applying very elaborate sample preparation including multiple extraction steps, distillation, and concentration.

As contaminations are considered as by-products of synthesis of the tetrazol moiety Buschmann and Holzgrabe [23] suggest the analyses of other tetrazol sartanes for NDMA (candesartan, irbesartan, losartan, olmesartan, Fig. 5a). The synthesis of these four APIs also implies the use of azidotributyltin(IV) or tributyltin chloride and  $\text{NaN}_3$ . In addition, the biphenyl coupling (Suzuki-reaction) is also mentioned in the losartan potassium monograph of Ph.Eur. [5]. Consequently, Europe and USA already expanded their risk assessment also to these drugs. During analyses of further sartan preparations in Official Medicines Control Laboratories of the General European Network NDEA was detected in preparations containing losartan from Hetero Labs [218].

The list of APIs may be extended by tetrazol compounds out of other therapeutic classes. Tetrazol formation of several cephalosporines as well as alfentanil and clostazol may utilize azides as well. Considering the above mentioned quenching process with  $\text{NaNO}_2$ , the formation of NDMA may occur in their synthesis as well if DMF is used as solvent. Chemical structures of examples are shown in Fig. 5b. Furthermore, the synthesis of multiple other APIs involves azides or nitrite in decomposition (i.e. metronidazole), for cleavage to yield low molecular weight heparines, or for use in activation of carboxylic acids, oxazolin opening, desulfurination, etc. (chemical structures of API examples in Fig. 5c). Furthermore, protein chemistry uses carbonic acid azides and thus, chemically synthesized proteins are also candidates for closer investigations of contaminations with NDMA.

Further APIs have been reported as known or suspect precursors of NDMA [39–42,44,45,48,149,150,219,220]: aminopyramine, amitriptyline, azithromycine, benzalkonium chloride, carboxamine, chloramphenicol, chlorphenamine (chlorpheniramine), chlorpromazine, chlorprothixene, chlortetracycline, citalopram, clarithromycin, clomipramine, dimenhydrinate, diltiazem, *N,N*-diethyltoluamide (DEET), diphenhydramine, doxepin, doxylamine, erythromycin, escitalopram, imipramine, meropenem, metformin, methapyrilene, methyl orange, methylthioninium chloride (methylene blue), mifepristone, minocycline, nizatidine, oleanandomycin, oxytetracycline, piramidon (dimethylaminophenazone), promazine, propoxyphene, quinupristin, ranitidine, roxithromycin, spiramycin, sumatriptan, trimipramine, tetracycline, tramadol, and venlafaxine.

Especially ranitidine is reported to show very high molar conversion to NDMA in oxidizing conditions [44,150]. Using ozone for



**Fig. 5.** Chemical structures of further APIs (examples) that utilize azides or nitrite in synthesis: (a) tetrazol sartanes, (b) other APIs with tetrazol moiety, (c) further APIs with different structures.

oxidation of ranitidine Lv et al. [149] found dimethylamine and NDMA as products. It may therefore be speculated that exposure to air and light may also lead to the formation of NDMA during API storage.

Interestingly, in Ph.Eur. only the monograph for irbesartan mentions purity testing for azide, and only very few tests for N-nitroso compounds are listed, e.g. for N-nitrosodiethanolamine (trolamine monograph, GC-CLD after silylation, cut-off 24 ppb) or dalteparin [3]. No test for NDMA is currently recommended.

Furthermore, Dawson et al. found several drug formulations with NDMA contaminations up to 12 ppb, even if the API in the formulation did not contain precursors for the observed nitrosamines [42].

According to the above mentioned we suggest to include a method for N-nitrosamine testing in the general chapters of pharmacopoeias. Due to the broad spectrum of potential APIs an inclusion in the general monograph "substances for pharmaceutical use" of Ph.Eur. is suggested.

As recommended in the ICH Guideline M7 potential carcinogenic compounds should be controlled if "reasonably expected in the final drug substance or product" [221]. This document also explicitly mentions N-nitroso compounds. Further toxicological considerations have been published and recently also focused on the assessment of NDMA as drug impurities. Reasonable acceptance limits have to be proposed on these basis in the near future.

## References

- [1] U.S. Food and Drug Administration, FDA Updates on Valsartan Recalls, U.S. Food and Drug Administration, 2018.
- [2] J. Leclerc, Recall of NDMA-contaminated pseudogeneric valsartan; best generics finally no better than others? *Can. J. Cardiol.* (2018).
- [3] Council of Europe, European Pharmacopoeia, Council of Europe, Strasbourg, 2018.
- [4] The United States pharmacopeial convention, United States Pharmacopoeia-USP 41 NF36, The United States pharmacopeial convention, Inc., Rockville, 2018.
- [5] F. Bracher, P. Heisig, P. Langguth, E. Mutschler, G. Rücker, T. Schirmeister, G.K.E. Scriba, E. Stahl-Biskup, R. Troschütz, *Arzneibuch-Kommentar*, 1. Aufl. inkl. 58. Akt.Ifg ed., Wissenschaftliche Verlagsgesellschaft Stuttgart, 2018.

- [6] S. Ghosh, A.S. Kumar, R. Soundararajan, G.N. Mehta, Improved synthesis of valsartan via nucleophilic aromatic substitution on aryloxazoline, *Synth. Commun.* 39 (2009) 3880–3887.
- [7] N.S. Kumar, S.B. Reddy, B.K. Sinha, K. Mukkanti, R. Dandala, New and improved manufacturing process for valsartan, *Org. Process Res. Dev.* 13 (2009) 1185–1189.
- [8] S. Ghosh, A.S. Kumar, G.N. Mehta, A short and efficient synthesis of valsartan via a Negishi reaction, *Beilstein J. Org. Chem.* 6 (2010) 27.
- [9] S. Ghosh, A.S. Kumar, G.N. Mehta, Convenient synthesis of Valsartan via a Suzuki reaction, *J. Chem. Res.* 34 (2010) 191–193.
- [10] M. Seki, Highly efficient catalytic system for C-H activation: a practical approach to angiotensin II receptor blockers, *ACS Catal.* 1 (2011) 607–610.
- [11] G.X. Wang, B.P. Sun, C.H. Peng, An improved synthesis of valsartan, *Org. Process Res. Dev.* 15 (2011) 986–988.
- [12] S. Ambati, H.R. Penikelapati, T.V. Maruthikumar, N.B. Ambati, Alternative synthesis of valsartan via Negishi coupling, *Pharma Chem.* 3 (2011) 13–17.
- [13] M. Seki, M. Nagahama, Synthesis of angiotensin II receptor blockers by means of a catalytic system for C-H activation, *J. Org. Chem.* 76 (2011) 10198–10206.
- [14] H.R. Penikelapati, S. Ambati, T.V. Maruthikumar, N.B. Ambati, New and improved synthesis of valsartan: an antihypertensive drug, *Res. J. Pharm. Biol. Chem. Sci.* 2 (2011) 632–639.
- [15] S. Aalla, G. Gillia, Y. Bojja, R.R. Anumula, P.R. Vummenthala, P.R. Padi, An efficient and telescopic process for Valsartan, an angiotensin II receptor blocker, *Org. Process Res. Dev.* 16 (2012) 682–686.
- [16] R.N. Patel, D.S. Patel, R.B. Patel, K.S. Patel, A novel and industrial approach for the synthesis of valsartan, *Heterocycl. Lett.* 3 (2013) 513–518.
- [17] A. Nagaki, K. Hirose, O. Tonomura, S. Taniguchi, T. Taga, S. Hasebe, N. Ishizuka, J. Yoshida, Design of a numbering-up system of monolithic microreactors and its application to synthesis of a key intermediate of valsartan, *Org. Process Res. Dev.* 20 (2016) 687–691.
- [18] J. Hubrich, L. Ackermann, Amino acid ligands for ruthenium(II)-catalyzed C-H arylation of aryltetrazoles with chlorides: expedient access to antihypertension drugs, *European J. Org. Chem.* 2016 (2016) 3700–3704.
- [19] J. Zou, Y. Yang, W. Wang, Synthesis of Valsartan, Beijing Second Pharmaceutical Co., Ltd., Peop. Rep. China, 2009, pp. 8pp.
- [20] S. Jain, R.S. Shekhawat, A.K. Tyagi, A. Agarwal, Process for the Production of Sartans With High Purity, Jubilant Life Sciences Limited, India, 2012, pp. 51pp.
- [21] Y. Wang, G. Zheng, G. Cai, B. Chen, H. Li, Process for Preparation of Valsartan, Zhejiang Hisun Pharmaceutical Co., Ltd., Peop. Rep. China, 2009, pp. 11pp.
- [22] Z. Xiaoren, S. Nianping, Z. Wenling, W. Peng, Improved Method for Preparing Tetrazole for Valsartan, Zhejiang Huaihai Pharmaceutical Co., Ltd., 2014, pp. 11pp.
- [23] H. Buschmann, U. Holzgrabe, NDMA in valsartan, *Apoth.* 158 (2018) 22–26.
- [24] M. Abdel-Tawab, R. Gröner, T. Kopp, J. Meins, J. Wübert, Tablettent, ZL findet NDMA in, *Pharmazeutische Zeitung* 30 (2018) (2018) 14–16.
- [25] W.A. Mitch, J.O. Sharp, R.R. Trussell, R.L. Valentine, L. Alvarez-Cohen, D.L. Sedlak, N-nitrosodimethylamine (NDMA) as a drinking water contaminant: a review, *Environ. Eng. Sci.* 20 (2003) 389–404.
- [26] US Food and Drug Administration, Dimethylnitrosamine in Malt Beverages, 2005.
- [27] S.D. Richardson, C. Postigo, Drinking water disinfection by-products, in: Emerging Organic Contaminants and Human Health, Springer, 2011, pp. 93–137.
- [28] World Health Organization, Guidelines for Drinking-water Quality, 2017.
- [29] U.S. Environmental Protection Agency, Technical Fact Sheet – N-Nitroso-dimethylamine, NDMA, 2014.
- [30] World Health Organization, N-Nitrosodimethylamine In Drinking-water. Background Document for Preparation of WHO Guidelines for Drinking-water Quality, in: Geneva, 2008.
- [31] International Agency for Research on Cancer, IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans - Some N-Nitroso Compounds, Lyon, 1978.
- [32] P. Andrezewski, B. Kasprzyk-Hordern, J. Nawrocki, The hazard of N-nitrosodimethylamine (NDMA) formation during water disinfection with strong oxidants, *Desalination* 176 (2005) 37–45.
- [33] R. Preussmann, Carcinogenic N-nitroso compounds and their environmental significance, *Naturwissenschaften* 71 (1984) 25–30.
- [34] US Food and Drug Administration, GC/MS Headspace Method for Detection of NDMA in Valsartan Drug Substance, 2018, <https://www.fda.gov/downloads/Drugs/DrugSafety/UCM618053.pdf>.
- [35] Council of Europe, Ad-hoc Projects of the OMCL Network - Methods for Determination of NDMA and NDEA in Sartans, 2018.
- [36] Public Analyst's Laboratory, Determination of NDMA (HS-GC-MS), Galway, Ireland, 2018, <https://www.edqm.eu/sites/default/files/omcl-ndma-method-palg-ie-september2018.pdf>.
- [37] O. el-Atma, B. Gutsche, Test method for the determination of NDMA and NDEA by LC-MS/MS in sartan containing film coated tablets, in: *Chemisches Und Veterinäruntersuchungsamt Karlsruhe*, 2018, Karlsruhe.
- [38] French National Agency for Medicines and Health Products Safety Laboratory Controls Devision, Determination of NDMA in Valsartan Active Substances and Finished Products by HPLC/UV, 2018, <https://www.edqm.eu/sites/default/files/omcl-method-determination-ndma-valsartan-an-sm-september2018.pdf>.
- [39] M. Castegnaro, B. Pignatelli, E.A. Walker, Analysis of volatile N-nitrosamines in commercial drugs, *Food Cosmet. Toxicol.* 19 (1981) 489–491.
- [40] G. Eisenbrand, B. Spiegelhalder, J. Kann, R. Klein, R. Preussmann, Carcinogenic N-nitrosodimethylamine as a contamination in drugs containing 4-dimethylamino-2,3-dimethyl-1-phenyl-3-pyrrololin-5-one (amidopyrine, aminophenazonate), *Arzneimittel-Forschung* 29 (1979) 867–869.
- [41] P. Taylor, P. Braddock, D. Carter, The analysis of N-nitrosodimethylamine in antihistamines and cough/cold preparations, *IARC Sci. Publ.* (1980) 575–587.
- [42] B.A. Dawson, R.C. Lawrence, Analysis of selected drug formulations for volatile nitrosamines, *J. Assoc. Off. Anal. Chem.* 70 (1987) 554–556.
- [43] I.S. Krull, U. Goff, A. Silvergleid, D.H. Fine, N-Nitroso compound contaminants in prescription and nonprescription drugs, *ArzneimittelForschung* 29 (1979) 870–874.
- [44] R. Shen, S.A. Andrews, Demonstration of 20 pharmaceuticals and personal care products (PPCPs) as nitrosamine precursors during chloramine disinfection, *Water Res.* 45 (2011) 944–952.
- [45] S.L. Leavay-Roback, S.W. Krasner, I.M. Suffet, Veterinary antibiotics used in animal agriculture as NDMA precursors, *Chemosphere* 164 (2016) 330–338.
- [46] R.C. Cheng, C.J. Hwang, C. Andrews-Tate, Y.B. Guo, S. Carr, I.H. Suffet, Alternative methods for the analysis of NDMA and other nitrosamines in water, *J. Am. Water Works Assoc.* 98 (2006) 82–96.
- [47] X. Wang, H. Yang, B. Zhou, X. Wang, Y. Xie, Effect of oxidation on amine-based pharmaceutical degradation and N-Nitrosodimethylamine formation, *Water Res.* 87 (2015) 403–411.
- [48] J. Lv, N. Li, Characterization of seven psychoactive pharmaceuticals as N-nitrosodimethylamine precursors during free chlorine and chlorine dioxide chlorination processes, *J. Chem. Technol. Biotechnol.* (2018).
- [49] C. Crews, The determination of N-nitrosamines in food, *Qual. Assur. Saf. Crop. Foods* 2 (2010) 2–12.
- [50] M.T. Empl, P. Kammerer, R. Ulrich, J.F. Joseph, M.K. Parr, I. Willenberg, N.H. Schebb, W. Baumgartner, E. Rohrdanz, C. Steffen, P. Steinberg, The influence of chronic L-carnitine supplementation on the formation of preneoplastic and atherosclerotic lesions in the colon and aorta of male F344 rats, *Arch. Toxicol.* 89 (2015) 2079–2087.
- [51] B. Jurado-Sánchez, E. Ballesteros, M. Gallego, Screening of N-nitrosamines in tap and swimming pool waters using fast gas chromatography, *J. Sep. Sci.* 33 (2010) 610–616.
- [52] T.H. Seyler, J.G. Kim, J.A. Hodgson, E.A. Cowan, B.C. Blount, L. Wang, Quantitation of urinary volatile nitrosamines from exposure to tobacco smoke, *J. Anal. Toxicol.* 37 (2013) 195–202.
- [53] J.W. Munch, M.V. Bassett, Method 521. Determination of Nitrosamines in Drinking Water by Solid-phase Extraction and Capillary Column Gas Chromatography With Large Volume Injection and Chemical Ionization Tandem Mass Spectrometry (MS/MS), U. S. Environmental Protection Agency, Cincinnati, Ohio, 2004.
- [54] B. Jurado-Sánchez, E. Ballesteros, M. Gallego, Comparison of the sensitivities of seven N-nitrosamines in pre-screened waters using an automated preconcentration system and gas chromatography with different detectors, *J. Chromatogr. A* 1154 (2007) 66–73.
- [55] S.F. Aygun, A. Uyanik, B. Batı, Adsorption of N-nitrosodiethylamine and N-nitrosodimethylamine on activated carbon: a pre-concentration procedure for gas chromatographic analysis, *Microchim. Ichnoanal. Acta* 146 (2004) 279–283.
- [56] M. Al-Kaseem, Z. Al-Assaf, F. Karabeet, Development and validation of GC-FID method for the determination of volatile N-nitrosamines in meat, *Int. J. Pharm. Sci. Rev. Res.* 25 (2014) 59–64.
- [57] S.F. Cooper, C. Lemoyne, D. Gauvreau, Identification and quantitation of N-nitrosamines in human postmortem organs, *J. Anal. Toxicol.* 11 (1987) 12–18.
- [58] N.P. Sen, Gas-liquid chromatographic determination of dimethylnitrosamine as dimethylnitramine at picogram levels, *J. Chromatogr. A* 51 (1970) 301–304.
- [59] G.A. Reineccius, S.T. Coulter, Examination of nonfat dry milk for the presence of nitrosamines, *J. Dairy Sci.* 55 (1972) 1574–1576.
- [60] T. Alliston, G. Cox, R. Kirk, The determination of steam-volatile N-nitrosamines in foodstuffs by formation of electron-capturing derivatives from electrochemically derived amines, *Analyst* 97 (1972) 915–920.
- [61] Fiddler, The occurrence and determination of N-nitroso compounds, *Toxicol. Appl. Pharmacol.* 31 (1975) 352–360.
- [62] B. Kenessov, Y. Sailaukhanuly, J.A. Koziel, L. Carlsen, M. Nauryzbayev, GC-MS and GC-NPD determination of formaldehyde dimethylhydrazone in water using SPME, *Chromatographia* 73 (2011) 123–128.
- [63] F. Reche, M.C. Garrigos, M.L. Marin, A. Canto, A. Jimenez, Optimization of parameters for the supercritical fluid extraction in the determination of N-nitrosamines in rubbers, *J. Chromatogr. A* 963 (2002) 419–426.
- [64] K. Takatsuki, T. Kikuchi, Determination of N-nitrosodimethylamine in fish products using gas chromatography with nitrogen–phosphorus detection, *J. Chromatogr. A* 508 (1990) 357–362.
- [65] J.E. Grebel, I.H. Mel Suffet, Nitrogen-phosphorus detection and nitrogen chemiluminescence detection of volatile nitrosamines in water matrices: optimization and performance comparison, *J. Chromatogr. A* 1175 (2007) 141–144.
- [66] J.E. Grebel, C.C. Young, I.H. Suffet, Solid-phase microextraction of N-nitrosamines, *J. Chromatogr. A* 1117 (2006) 11–18.

- [67] D.H. Fine, F. Rufeh, D. Lieb, Group analysis of volatile and non-volatile N-nitroso compounds, *Nature* 247 (1974) 309–310.
- [68] D.H. Fine, F. Rufeh, D. Lieb, D.P. Rounbehler, Description of the thermal energy analyzer (TEA) for trace determination of volatile and nonvolatile N-nitroso compounds, *Anal. Chem.* 47 (1975) 1188–1191.
- [69] G. Telling, The determination of N-nitrosamines in foods and cosmetics, *Trac Trends Anal. Chem.* 1 (1982) 277–280.
- [70] M.W. Byun, H.J. Ahn, J.H. Kim, J.W. Lee, H.S. Yook, S.B. Han, Determination of volatile N-nitrosamines in irradiated fermented sausage by gas chromatography coupled to a thermal energy analyzer, *J. Chromatogr. A* 1054 (2004) 403–407.
- [71] R.J. Maxwell, J.W. Pensabene, W. Fiddler, Multiresidue recovery at Ppb levels of 10 nitrosamines from frankfurters by supercritical-fluid extraction, *J. Chromatogr. Sci.* 31 (1993) 212–215.
- [72] S.Y. Choi, M.J. Chung, N.J. Sung, Volatile N-nitrosamine inhibition after intake Korean green tea and Maesil (*Prunus mume* Sieb. et ZACC) extracts with an amine-rich diet in subjects ingesting nitrate, *Food Chem. Toxicol.* 40 (2002) 949–957.
- [73] J.M. Fajen, G.A. Carson, D.P. Rounbehler, T.Y. Fan, R. Vita, U.E. Goff, M.H. Wolf, G.S. Edwards, D.H. Fine, V. Reinhold, K. Biemann, N-nitrosamines in the rubber and tire industry, *Science* 205 (1979) 1262–1264.
- [74] F. Shahidi, R.B. Pegg, N.P. Sen, Absence of volatile N-nitrosamines in cooked nitrite-free cured muscle foods, *Meat Sci.* 37 (1994) 327–336.
- [75] S. Raoul, E. Gremaud, H. Biaudet, R.J. Turesky, Rapid solid-phase extraction method for the detection of volatile nitrosamines in food, *J. Agric. Food Chem.* 45 (1997) 4706–4713.
- [76] D.C. Haverty, J.H. Hotchkiss, T. Fazio, Rapid determination of volatile N-nitrosamines in nonfat dry milk, *J. Dairy Sci.* 65 (1982) 182–185.
- [77] E.U. Goff, D.H. Fine, Analysis of volatile N-nitrosamines in alcoholic beverages, *Food Cosmet. Toxicol.* 17 (1979) 569–573.
- [78] M.J. Chung, S.H. Lee, N.J. Sung, Inhibitory effect of whole strawberries, garlic juice or kale juice on endogenous formation of N-nitrosodimethylamine in humans, *Cancer Lett.* 182 (2002) 1–10.
- [79] R. Andrade, F.G.R. Reyes, S. Rath, A method for the determination of volatile N-nitrosamines in food by HS-SPME-GC-TEA, *Food Chem.* 91 (2005) 173–179.
- [80] G. Telling, P. Dunnnett, The determination of N-nitrosodiethanolamine (NDELA) at trace levels in shampoos and skin creams by a simple, rapid colorimetric method, *Int. J. Cosmet. Sci.* 3 (1981) 241–248.
- [81] B.A. Tomkins, W.H. Griest, C.E. Higgins, Determination of N-Nitrosodimethylamine at Part-Per-Trillion Levels in Drinking Waters and Contaminated Groundwaters, *Anal. Chem.* 67 (1995) 4387–4395.
- [82] M.Z. Ozel, F. Gogus, S. Yagci, J.F. Hamilton, A.C. Lewis, Determination of volatile nitrosamines in various meat products using comprehensive gas chromatography–nitrogen chemiluminescence detection, *Food Chem.* Toxicol. 48 (2010) 3268–3273.
- [83] S.M. Billedieu, B.J. Miller, H.C. Thompson, N-nitrosamine analysis in beer using thermal-desorption injection coupled with Gc-tea, *J. Food Sci.* 53 (1988) 1696–1697.
- [84] S.R. Dunn, J.W. Pensabene, M.L. Simenhoff, Analysis of human blood for volatile N-nitrosamines by gas chromatography–chemiluminescence detection, *J. Chromatogr.* 377 (1986) 35–47.
- [85] W.H. Chen, C.Y. Wang, T.H. Huang, Formation and fates of nitrosamines and their formation potentials from a surface water source to drinking water treatment plants in Southern Taiwan, *Chemosphere* 161 (2016) 546–554.
- [86] D. Feng, L. Liu, L. Zhao, Q. Zhou, T. Tan, Evaluation of simulant migration of volatile nitrosamines from latex gloves and balloons by HS-SPME-GC-MS, *J. Chromatogr. Sci.* 50 (2012) 733–738.
- [87] H.K. Ju, H.W. Chung, H.S. Lee, J. Lim, J.H. Park, S.C. Lim, J.M. Kim, S.S. Hong, S.W. Kwon, Investigation of metabolite alteration in dimethylnitrosamine-induced liver fibrosis by GC-MS, *Bioanalysis* 5 (2013) 41–51.
- [88] S. Ventanas, D. Martín, M. Estévez, J. Ruiz, Analysis of volatile nitrosamines from a model system using SPME-DED at different temperatures and times of extraction, *Food Chem.* 99 (2006) 842–850.
- [89] A. Rakshit, S. Johri, Determination of N-nitrosodimethylamine in environmental aqueous samples by isotope-dilution GC/MS-SIM, *J. AOAC Int.* 84 (2001) 1413–1419.
- [90] J.A. McDonald, N.B. Harden, L.D. Nghiem, S.J. Khan, Analysis of N-nitrosamines in water by isotope dilution gas chromatography-electron ionisation tandem mass spectrometry, *Talanta* 99 (2012) 146–154.
- [91] S. Yurchenko, U. Molder, N-nitrosodimethylamine analysis in Estonian beer using positive-ion chemical ionization with gas chromatography mass spectrometry, *Food Chem.* 89 (2005) 455–463.
- [92] F.J. Lona-Ramirez, G. Gonzalez-Alatorre, V. Rico-Ramirez, M.C. Perez-Perez, E.O. Castrejon-Gonzalez, Gas chromatography/mass spectrometry for the determination of nitrosamines in red wine, *Food Chem.* 196 (2016) 1131–1136.
- [93] D. Hanigan, J. Zhang, P. Herckes, S.W. Krasner, C. Chen, P. Westerhoff, Adsorption of N-nitrosodimethylamine precursors by powdered and granular activated carbon, *Environ. Sci. Technol.* 46 (2012) 12630–12639.
- [94] V.G. Amelin, D.K. Lavrukhan, Combination of microwave heating extraction and dispersive liquid–liquid microextraction for the determination of nitrosamines in foods using gas-liquid chromatography with a mass-spectrometric detector, *J. Anal. Chem.* 71 (2016) 359–364.
- [95] H. Dong, X. Guo, Y. Xian, H. Luo, B. Wang, Y. Wu, A salting out-acetonitrile homogeneous extraction coupled with gas chromatography-mass spectrometry method for the simultaneous determination of thirteen N-nitrosamines in skin care cosmetics, *J. Chromatogr. A* 1422 (2015) 82–88.
- [96] H. Ramezani, H. Hosseini, M. Kamankesh, V. Ghasemzadeh-Mohammadi, A. Mohammadi, Rapid determination of nitrosamines in sausage and salami using microwave-assisted extraction and dispersive liquid–liquid microextraction followed by gas chromatography–mass spectrometry, *Eur. Food Res. Technol.* 240 (2015) 441–450.
- [97] N. Campillo, P. Viñas, N. Martínez-Castillo, M. Hernández-Córdoba, Determination of volatile nitrosamines in meat products by microwave-assisted extraction and dispersive liquid–liquid microextraction coupled to gas chromatography–mass spectrometry, *J. Chromatogr. A* 1218 (2011) 1815–1821.
- [98] J.W.A. Charrois, M.W. Arend, K.L. Froese, S.E. Hruedy, Detecting N-nitrosamines in drinking water at nanogram per liter levels using ammonia positive chemical ionization, *Environ. Sci. Technol.* 38 (2004) 4835–4841.
- [99] J.W. Munch, M.V. Bassett, Method development for the analysis of N-nitrosodimethylamine and other N-nitrosamines in drinking water at low nanogram/liter concentrations using solid-phase extraction and gas chromatography with chemical ionization tandem mass spectrometry, *J. AOAC Int.* 89 (2006) 486–497.
- [100] J.C. Holady, R.A. Trenholm, S.A. Snyder, Use of automated solid-phase extraction and GC-MS/MS to evaluate nitrosamines in water matrices, *Am. Lab.* 44 (2012) 25–30.
- [101] A. Llop, F. Borrull, E. Pocurull, Fully automated determination of N-nitrosamines in environmental waters by headspace solid-phase microextraction followed by GC-MS-MS, *J. Sep. Sci.* 33 (2010) 3692–3700.
- [102] A. Llop, F. Borrull, E. Pocurull, Pressurised hot water extraction followed by headspace solid-phase microextraction and gas chromatography-tandem mass spectrometry for the determination of N-nitrosamines in sewage sludge, *Talanta* 88 (2012) 284–289.
- [103] H.-W. Hung, T.-F. Lin, C.-H. Chiu, Y.-C. Chang, T.-Y. Hsieh, Trace analysis of n-nitrosamines in water using solid-phase microextraction coupled with gas chromatograph–tandem mass spectrometry, *Water Air Soil Pollut.* 213 (2010) 459–469.
- [104] R. Pozzi, P. Bocchini, F. Pinelli, G.C. Galletti, Determination of nitrosamines in water by gas chromatography/chemical ionization/selective ion trapping mass spectrometry, *J. Chromatogr. A* 1218 (2011) 1808–1814.
- [105] A. Sannino, L. Bolzon, GC/CI-MS/MS method for the identification and quantification of volatile N-nitrosamines in meat products, *Food Chem.* 141 (2013) 3925–3930.
- [106] J.E. Park, J.E. Seo, J.Y. Lee, H. Kwon, Distribution of seven N-Nitrosamines in food, *Toxicol. Res.* 31 (2015) 279–288.
- [107] S. Yoon, N. Nakada, H. Tanaka, A new method for quantifying N-nitrosamines in wastewater samples by gas chromatography–triple quadrupole mass spectrometry, *Talanta* 97 (2012) 256–261.
- [108] J.B. Plomley, C.J. Koester, R.E. March, Determination of N-nitrosodimethylamine in complex environmental matrices by quadrupole ion storage tandem mass-spectrometry enhanced by unidirectional ion ejection, *Anal. Chem.* 66 (1994) 4437–4443.
- [109] Y. Hong, K.H. Kim, B.I. Sang, H. Kim, Simple quantification method for N-nitrosamines in atmospheric particulates based on facile pretreatment and GC-MS/MS, *Environ. Pollut.* 226 (2017) 324–334.
- [110] F. Lv, J. Guo, F. Yu, T. Zhang, S. Zhang, H. Cui, X. Liu, L. Chen, L. Liu, S. Liu, F. Xie, Determination of nine volatile N-nitrosamines in tobacco and smokeless tobacco products by dispersive solid-phase extraction with gas chromatography and tandem mass spectrometry, *J. Sep. Sci.* 39 (2016) 2123–2128.
- [111] Z. Wang, M. Zhai, X. Xia, M. Yang, T. Han, M. Huang, A simple method for monitoring eight N-nitrosamines in Beef Jerky's by gas chromatography-tandem mass spectrometry with one-step treatment coupled to active carbon solid-phase extraction, *Food Anal. Methods* 11 (2018) 933–938.
- [112] S.J. Lehotay, Y. Sapozhnikova, L. Han, J.J. Johnston, Analysis of nitrosamines in cooked bacon by QuEChERS sample preparation and gas chromatography-tandem mass spectrometry with backflushing, *J. Agric. Food Chem.* 63 (2015) 10341–10351.
- [113] Y. Qiu, J.-H. Chen, W. Yu, P. Wang, M. Rong, H. Deng, Contamination of Chinese salted fish with volatile N-nitrosamines as determined by QuEChERS and gas chromatography-tandem mass spectrometry, *Food Chem.* 232 (2017) 763–769.
- [114] W. Chen, X. Li, H. Huang, X. Zhu, X. Jiang, Y. Zhang, K. Cen, L. Zhao, X. Liu, S. Qi, Comparison of gas chromatography-mass spectrometry and gas chromatography-tandem mass spectrometry with electron ionization for determination of N-nitrosamines in environmental water, *Chemosphere* 168 (2017) 1400–1410.
- [115] M.J. Farre, S. Insa, J. Mamo, D. Barcelo, Determination of 15 N-nitrosodimethylamine precursors in different water matrices by automated on-line solid-phase extraction ultra-high-performance-liquid chromatography tandem mass spectrometry, *J. Chromatogr. A* 1458 (2016) 99–111.
- [116] M. Allinson, K. Kadokami, F. Shiraishi, D. Nakajima, J. Zhang, A. Knight, S.R. Gray, P.J. Scales, G. Allinson, Wastewater recycling in Antarctica:

- performance assessment of an advanced water treatment plant in removing trace organic chemicals, *J. Environ. Manage.* 224 (2018) 122–129.
- [117] Q. Ma, H.-W. Xi, C. Wang, H. Bai, G.-C. Xi, N. Su, L.-Y. Xu, J.-B. Wang, Determination of ten volatile nitrosamines in cosmetics by gas chromatography tandem mass spectrometry, *Chinese J. Anal. Chem.* 39 (2011) 1201–1207.
- [118] C. Planas, O. Palacios, F. Ventura, J. Rivera, J. Caixach, Analysis of nitrosamines in water by automated SPE and isotope dilution GC/HRMS occurrence in the different steps of a drinking water treatment plant, and in chlorinated samples from a reservoir and a sewage treatment plant effluent, *Talanta* 76 (2008) 906–913.
- [119] P.J. Groenen, R.J. Jonk, C. van Ingen, M.C. ten Noever de Brauw, Determination of eight volatile nitrosamines in thirty cured meat products with capillary gas chromatography-high-resolution mass spectrometry: the presence of nitrosodimethylamine and the absence of nitrosopyrrolidine, *IARC Sci. Publ.* (1976) 321–331.
- [120] X.F. Zeng, W.D. Bai, Y.P. Xian, H. Dong, D.H. Luo, Application of QuEChERS-based purification coupled with isotope dilution gas chromatography-mass spectrometry method for the determination of N-nitrosamines in soy sauce, *Anal Methods-Uk* 8 (2016) 5248–5254.
- [121] S. Yurchenko, U. Molder, Volatile N-nitrosamines in various fish products, *Food Chem.* 96 (2006) 325–333.
- [122] G. Yeh, J.D. Ebeler, S.E. Ebeler, Analysis of nitrosamines in foods and beverages, *Chromatogr. Sci. Ser.* 77 (1998) 77–92.
- [123] J.A. Incavo, M.A. Schafer, Simplified method for the determination of N-nitrosamines in rubber vulcanizates, *Anal. Chim. Acta* 557 (2006) 256–261.
- [124] D. Orazbayeva, B. Kenessov, E. Psillakis, D. Nassirova, M. Bektassov, Determination of transformation products of unsymmetrical dimethylhydrazine in water using vacuum-assisted headspace solid-phase microextraction, *J. Chromatogr. A* 1555 (2018) 30–36.
- [125] B.N. Kenessov, J.A. Koziel, T. Grotenhuis, L. Carlsen, Screening of transformation products in soils contaminated with unsymmetrical dimethylhydrazine using headspace SPME and GC-MS, *Anal. Chim. Acta* 674 (2010) 32–39.
- [126] D.M. Perez, G.G. Alatorre, E.B. Alvarez, E.E. Silva, J.F.J. Alvarado, Solid-phase microextraction of N-nitrosodimethylamine in beer, *Food Chem.* 107 (2008) 1348–1352.
- [127] N.P. Sen, S.W. Seaman, B.D. Page, Rapid semi-quantitative estimation of N-nitrosodibutylamine and N-nitrosodibenzylamine in smoked hams by solid-phase microextraction followed by gas chromatography-thermal energy analysis, *J. Chromatogr. A* 788 (1997) 131–140.
- [128] C.C. Fan, T.F. Lin, N-nitrosamines in drinking water and beer: detection and risk assessment, *Chemosphere* 200 (2018) 48–56.
- [129] N.R. Choi, Y.P. Kim, W.H. Ji, G.S. Hwang, Y.G. Ahn, Identification and quantification of seven volatile n-nitrosamines in cosmetics using gas chromatography/chemical ionization-mass spectrometry coupled with head space-solid phase microextraction, *Talanta* 148 (2016) 69–74.
- [130] T.H. Sucipto, G. Supriyanto, Y. Raharjo, Analysis of N-nitrosodiprophylamines carcinogenic compound in meat-processing using headspace-single drop microextraction-gas chromatography-flame ionization detector (HS-SDME-GC-FID), *Iptek J. Proc. Ser.* 2 (2016).
- [131] B.A. Tomkins, W.H. Griest, Determinations of N-nitrosodimethylamine at part-per-trillion concentrations in contaminated groundwaters and drinking waters featuring carbon-based membrane extraction disks, *Anal. Chem.* 68 (1996) 2533–2540.
- [132] Agilent Technologies, Nitrosamine analysis by gas chromatography and agilent 255 nitrogen chemiluminescence detector (NCD), in: A.T. Inc (Ed.), Technical Overview, 2007, pp. 1–2, Wilmington, DE, Santa Clara, CA.
- [133] E.G. Cowley, J.R. Partington, Dielectric polarization. IX. The dipole moments of some nitrosoamines, p-nitrosophenol, ethylaniline, hydrazobenzene and benzaldehyde phenylhydrazone, *J. Chem. Soc.* (1933) 1255–1257.
- [134] W.X. Li, N. Chen, Y.G. Zhao, W.Q. Guo, N. Muhammd, Y. Zhu, Z.P. Huang, Online coupling of tandem liquid-phase extraction with HPLC-UV for the determination of trace N-nitrosamines in food products, *Anal. Methods-Uk* 10 (2018) 1733–1739.
- [135] M. Al-Kaseem, Z. Al-Assaf, F. Karabheet, A. Rapid, Validated RP-HPLC method for the determination of seven volatile N-nitrosamines in meat, *Pharmacol. Pharm.* 05 (2014) 298–308.
- [136] W. Wang, S. Ren, H. Zhang, J. Yu, W. An, J. Hu, M. Yang, Occurrence of nine nitrosamines and secondary amines in source water and drinking water: potential of secondary amines as nitrosamine precursors, *Water Res.* 45 (2011) 4930–4938.
- [137] E. Topuz, E. Aydin, E. Pehlivanoglu-Mantas, A. Practical, LC-MS/MS method for the detection of NDMA at nanogram per liter concentrations in multiple water matrices, *Water Air Soil Pollut.* 223 (2012) 5793–5802.
- [138] J.L. Roux, H. Gallard, J.P. Croue, S. Papot, M. Debordet, NDMA formation by chloramination of ranitidine: kinetics and mechanism, *Environ. Sci. Technol.* 46 (2012) 11095–11103.
- [139] M. Asami, M. Oya, K. Kosaka, A nationwide survey of NDMA in raw and drinking water in Japan, *Sci. Total Environ.* 407 (2009) 3540–3545.
- [140] M.H. Plumlee, M. Lopez-Mesas, A. Heidlberger, K.P. Ishida, M. Reinhard, N-nitrosodimethylamine (NDMA) removal by reverse osmosis and UV treatment and analysis via LC-MS/MS, *Water Res.* 42 (2008) 347–355.
- [141] C. Ripolles, E. Pitarch, J.V. Sancho, F.J. Lopez, F. Hernandez, Determination of eight nitrosamines in water at the ng L<sup>-1</sup> levels by liquid chromatography coupled to atmospheric pressure chemical ionization tandem mass spectrometry, *Anal. Chim. Acta* 702 (2011) 62–71.
- [142] Y. Kadmi, L. Favier, I. Soutrel, M. Lemasse, D. Wolbert, Ultratrace-level determination of N-nitrosodimethylamine, N-nitrosodimethylamine, and N-nitrosomorpholine in waters by solid-phase extraction followed by liquid chromatography-tandem mass spectrometry, *Cent. Eur. J. Chem.* 12 (2014) 928–936.
- [143] S.S. Herrmann, L. Duedahl-Olesen, K. Granby, Simultaneous determination of volatile and non-volatile nitrosamines in processed meat products by liquid chromatography tandem mass spectrometry using atmospheric pressure chemical ionisation and electrospray ionisation, *J. Chromatogr. A* 1330 (2014) 20–29.
- [144] N.Y. Park, W. Jung, Y. Kho, Analysis method of N-nitrosamines in human urine by LC-MS/MS system, *J. Korean Chem. Soc.* 61 (2017) 51–56.
- [145] J.H. Lee, S.U. Lee, J.E. Oh, Analysis of nine nitrosamines in water by combining automated solid-phase extraction with high-performance liquid chromatography-atmospheric pressure chemical ionisation tandem mass spectrometry, *Int. J. Environ. Anal. Chem.* 93 (2013) 1261–1273.
- [146] J.H. Lee, J.E. Oh, A comprehensive survey on the occurrence and fate of nitrosamines in sewage treatment plants and water environment, *Sci. Total Environ.* 556 (2016) 330–337.
- [147] C.W. Hu, Y.M. Shih, H.H. Liu, Y.C. Chiang, C.M. Chen, M.R. Chao, Elevated urinary levels of carcinogenic N-nitrosamines in patients with urinary tract infections measured by isotope dilution online SPE LC-MS/MS, *J. Hazard. Mater.* 310 (2016) 207–216.
- [148] J. Lv, Y. Li, Y. Song, Reinvestigation on the ozonation of N-nitrosodimethylamine: Influencing factors and degradation mechanism, *Water Res.* 47 (2013) 4993–5002.
- [149] J. Lv, L. Wang, Y. Li, Characterization of N-nitrosodimethylamine formation from the ozonation of ranitidine, *J. Environ. Sci. (China)* 58 (2017) 116–126.
- [150] A. Zhang, Y. Li, Y. Song, J. Lv, J. Yang, Characterization of pharmaceuticals and personal care products as N-nitrosodimethylamine precursors during disinfection processes using free chlorine and chlorine dioxide, *J. Hazard. Mater.* 276 (2014) 499–509.
- [151] W. Wang, J. Hu, J. Yu, M. Yang, Determination of N-nitrosodimethylamine in drinking water by UPLC-MS/MS, *J. Environ. Sci. (China)* 22 (2010) 1508–1512.
- [152] W. Wang, J. Yu, W. An, M. Yang, Occurrence and profiling of multiple nitrosamines in source water and drinking water of China, *Sci. Total Environ.* 551–552 (2016) 489–495.
- [153] X. Wang, Z. Liu, C. Wang, Z. Ying, W. Fan, W. Yang, Occurrence and formation potential of nitrosamines in river water and ground water along the Songhua River, *China. J. Environ. Sci. (China)* 50 (2016) 65–71.
- [154] E. Bei, Y. Shu, S. Li, X. Liao, J. Wang, X. Zhang, C. Chen, S. Krasner, Occurrence of nitrosamines and their precursors in drinking water systems around mainland China, *Water Res.* 98 (2016) 168–175.
- [155] Y.Y. Zhao, J. Boyd, S.E. Hruday, X.F. Li, Characterization of new nitrosamines in drinking water using liquid chromatography tandem mass spectrometry, *Environ. Sci. Technol.* 40 (2006) 7636–7641.
- [156] A.D. Ngongang, S.V. Duy, S. Sauve, Analysis of nine N-nitrosamines using liquid chromatography-accurate mass high resolution-mass spectrometry on a Q-exactive instrument, *Anal. Methods-Uk* 7 (2015) 5748–5759.
- [157] M. Krauss, J. Hollender, Analysis of nitrosamines in wastewater: exploring the trace level quantification capabilities of a hybrid linear ion trap/orbitrap mass spectrometer, *Anal. Chem.* 80 (2008) 834–842.
- [158] Y.Y. Zhao, X. Liu, J.M. Boyd, F. Qin, J.J. Li, X.F. Li, Identification of N-nitrosamines in treated drinking water using nanoelectrospray ionization high-field asymmetric waveform ion mobility spectrometry with quadrupole time-of-flight mass spectrometry, *J. Chromatogr. Sci.* 47 (2009) 92–96.
- [159] I.S. Krull, E.U. Goff, G.G. Hoffman, D.H. Fine, Confirmatory methods for the thermal-energy determination of N-nitroso compounds at trace levels, *Anal. Chem.* 51 (1979) 1706–1709.
- [160] A. Tricker, M. Perkins, R. Massey, C. Bishop, P. Key, D. McWeeny, Incidence of some non-volatile N-nitroso compounds in cured meats, *Food Addit. Contam.* 1 (1984) 245–252.
- [161] P. Oettinger, F. Huffman, D. Fine, D. Lieb, Liquid chromatograph detector for trace analysis of non-volatile N-nitroso compounds, *Anal. Lett.* 8 (1975) 411–414.
- [162] R.N. Loeppky, Nitrosamine and N-nitroso compound chemistry and biochemistry, in: *Nitrosamines and Related N-Nitroso Compounds*, American Chemical Society, 1994, pp. 1–18.
- [163] T. Pérez-Ruiz, C. Martínez-Lozano, V. Tomás, J. Martín, Automated solid-phase extraction and high-performance liquid chromatographic determination of nitrosamines using post-column photolysis and tris(2,2'-bipyridyl) ruthenium(III) chemiluminescence, *J. Chromatogr. A* 1077 (2005) 49–56.
- [164] H. Kodamatani, S. Yamazaki, K. Saito, A. Amponsaa-Karikari, N. Kishikawa, N. Kuroda, T. Tomiyasu, Y. Komatsu, Highly sensitive method for determination of N-nitrosamines using high-performance liquid chromatography with online UV irradiation and luminol chemiluminescence detection, *J. Chromatogr. A* 1216 (2009) 92–98.
- [165] H. Kodamatani, H. Yamasaki, T. Sakaguchi, S. Itoh, Y. Iwaya, M. Saga, K. Saito, R. Kanzaki, T. Tomiyasu, Rapid method for monitoring N-nitrosodimethylamine in drinking water at the ng/L level without pre-concentration using high-performance liquid

- chromatography-chemiluminescence detection, *J. Chromatogr. A* 1460 (2016) 202–206.
- [166] S.H. Kim, J.H. Hotchkiss, Nonvolatile N-nitrosamides in dried squid, in: Nitrosamines and Related N-Nitroso Compounds, American Chemical Society, 1994, pp. 355–357.
- [167] R. Zhang, D.T. Hess, Z. Qian, A. Hausladen, F. Fonseca, R. Chaube, J.D. Reynolds, J.S. Stamler, Hemoglobin betaCys93 is essential for cardiovascular function and integrated response to hypoxia, *Proc. Natl. Acad. Sci. U. S. A.* 112 (2015) 6425–6430.
- [168] T. Fujioka, H. Takeuchi, H. Tanaka, L.D. Nghiem, K.P. Ishida, H. Kodamatani, A rapid and reliable technique for N-nitrosodimethylamine analysis in reclaimed water by HPLC-photochemical reaction-chemiluminescence, *Chemosphere* 161 (2016) 104–111.
- [169] X. Li, X. He, Y. Dong, L. Jia, Q. He, Analysis of N-nitrosodiethylamine by ion chromatography coupled with UV photolysis pretreatment, *J. Food Drug Anal.* 24 (2016) 311–315.
- [170] W. Cha, P. Fox, B. Nalinakumari, High-performance liquid chromatography with fluorescence detection for aqueous analysis of nanogram-level N-nitrosodimethylamine, *Anal. Chim. Acta* 566 (2006) 109–116.
- [171] L. Cardenes, J.H. Ayala, V. Gonzalez, A.M. Afonso, Fast microwave-assisted dansylation of N-nitrosamines. Analysis by high-performance liquid chromatography with fluorescence detection, *J. Chromatogr. A* 946 (2002) 133–140.
- [172] S. Lu, D. Wu, G. Li, Z. Lv, P. Gong, L. Xia, Z. Sun, G. Chen, X. Chen, J. You, Y. Wu, Facile and sensitive determination of N-nitrosamines in food samples by high-performance liquid chromatography via combining fluorescent labeling with dispersive liquid-liquid microextraction, *Food Chem.* 234 (2017) 408–415.
- [173] G.B. Cox, Estimation of volatile N-nitrosamines by high-performance liquid chromatography, *J. Chromatogr.* 83 (1973) 471–481.
- [174] L. Cardenes, J.H. Ayala, V. Gonzalez, A.M. Afonso, Determination of N-nitrosamines by HPTLC with fluorescence detection - use of non-ionic surfactants as enhancing agents, *Jpc-J. Planar Chromatogr.-Modern TLC* 15 (2002) 349–353.
- [175] N.P. Sen, C. Dalpe, A simple thin-layer chromatographic technique for the semi-quantitative determination of volatile nitrosamines in alcoholic beverages, *Analyst* 97 (1972) 216–220.
- [176] C. Fu, H. Xu, Z. Wang, Sensitive assay system for nitrosamines utilizing high-performance liquid chromatography with peroxyxalate chemiluminescence detection, *J. Chromatogr. A* 634 (1993) 221–227.
- [177] Z. Wang, H. Xu, C. Fu, Sensitive fluorescence detection of some nitrosamines by precolumn derivatization with dansyl chloride and high-performance liquid chromatography, *J. Chromatogr. A* 589 (1992) 349–352.
- [178] X. Ceto, C.P. Saint, C.W.K. Chow, N.H. Voelcker, B. Prieto-Simon, Electrochemical detection of N-nitrosodimethylamine using a molecular imprinted polymer, *Sensor Actuat B-Chem* 237 (2016) 613–620.
- [179] F. Breider, U. von Gunten, Quantification of total N-nitrosamine concentrations in aqueous samples via UV-photolysis and chemiluminescence detection of nitric oxide, *Anal. Chem.* 89 (2017) 1574–1582.
- [180] E. Luque-Perez, A. Rios, M. Valcarcel, Automated flow-injection spectrophotometric determination of nitrosamines in solid food samples, *Fresenius J. Anal. Chem.* 371 (2001) 891–895.
- [181] G. Eisenbrand, R. Preussmann, A new method for the colorimetric determination of nitrosamines after cleavage of the N-nitroso-group with hydrogen bromide in glacial acetic acid, *Arzneimittel-Forschung* 20 (1970) 1512–1517.
- [182] T.-Y. Fan, S.R. Tannenbaum, Automatic colorimetric determination of nitroso compounds, *J. Agric. Food Chem.* 19 (1971) 1267–1269.
- [183] D.L.H. Williams, Reagents effecting nitrosation, in: D.L.H. Williams (Ed.), Nitrosation Reactions and the Chemistry of Nitric Oxide, Elsevier Science, Amsterdam, 2004, pp. 1–34.
- [184] L. Walsh, P.W. Hastwell, P.O. Keenan, A.W. Knight, N. Billinton, R.M. Walmsley, Genetic modification and variations in solvent increase the sensitivity of the yeast RAD54-GFP genotoxicity assay, *Mutagenesis* 20 (2005) 317–327.
- [185] V.N. Bui, T.T. Nguyen, C.T. Mai, Y. Bettarel, T.Y. Hoang, T.T. Trinh, N.H. Truong, H.H. Chu, V.T. Nguyen, H.D. Nguyen, S. Wolff, Procarcinogens - determination and evaluation by yeast-based biosensor transformed with plasmids incorporating RAD54 reporter construct and cytochrome P450 genes, *PLoS One* 11 (2016), e0168721.
- [186] G. Eisenbrand, B. Spiegelhalder, C. Janzowski, J. Kann, R. Preussmann, Volatile and non-volatile N-nitroso compounds in foods and other environmental media, *IARC Sci. Publ.* (1978) 311–324.
- [187] W. Zhou, C. Chen, L. Lou, Q. Yang, L. Zhu, Formation potential of nine nitrosamines from corresponding secondary amines by chloramination, *Chemosphere* 95 (2014) 81–87.
- [188] G.C. Woods-Chabane, C.M. Glover, E.J. Marti, E.R.V. Dickenson, A novel assay to measure tertiary and quaternary amines in wastewater: an indicator for NDMA wastewater precursors, *Chemosphere* 179 (2017) 298–305.
- [189] S. Yoon, N. Nakada, H. Tanaka, A new method for quantifying N-nitrosamines in wastewater samples by gas chromatography-triple quadrupole mass spectrometry, *Talanta* 97 (2012) 256–261.
- [190] M. Lee, Y. Lee, F. Soltermann, U. von Gunten, Analysis of N-nitrosamines and other nitro(so) compounds in water by high-performance liquid chromatography with post-column UV photolysis/Griess reaction, *Water Res.* 47 (2013) 4893–4903.
- [191] S.C. Fu, S.H. Tzing, H.C. Chen, Y.C. Wang, W.H. Ding, Dispersive micro-solid phase extraction combined with gas chromatography-chemical ionization mass spectrometry for the determination of N-nitrosamines in swimming pool water samples, *Anal. Bioanal. Chem.* 402 (2012) 2209–2216.
- [192] M.C. Huang, H.C. Chen, S.C. Fu, W.H. Ding, Determination of volatile N-nitrosamines in meat products by microwave-assisted extraction coupled with dispersive micro solid-phase extraction and gas chromatography-chemical ionisation mass spectrometry, *Food Chem.* 138 (2013) 227–233.
- [193] M. Mutsuga, M. Yamaguchi, Y. Kawamura, Analysis of N-Nitrosamine migration from rubber teats and soothers, *Am. J. Analyt. Chem.* 04 (2013) 277–285.
- [194] S.-J. Park, M.-J. Jeong, S.-R. Park, J.C. Choi, H. Choi, M. Kim, Release of N-nitrosamines and N-nitrosatable substances from baby bottle teats and rubber kitchen tools in Korea, *Food Sci. Biotechnol.* (2018).
- [195] N.Y. Park, S. Kim, W. Jung, Y. Kho, Analysis of nitrosamines concentration in condom by using LC-MS/MS, *J. Korean Chem. Soc.* 62 (2018) 181–186.
- [196] J.A. Hodgson, T.H. Seyler, E. McGahee, S. Arnestin, L. Wang, A new automated method and sample data flow for analysis of volatile nitrosamines in human urine, *Am. J. Analyt. Chem.* 7 (2016) 165–178.
- [197] J.A. Hodgson, T.H. Seyler, L. Wang, Long-term stability of volatile nitrosamines in human urine, *J. Anal. Toxicol.* 40 (2016) 414–418.
- [198] M. Alyuz, S. Ata, E. Dinc, A chemometric optimization of method for determination of nitrosamines in gastric juices by GC-MS, *J. Pharm. Biomed. Anal.* 117 (2016) 26–36.
- [199] N. Ramirez, L. Vallecillos, A.C. Lewis, F. Borrull, R.M. Marce, J.F. Hamilton, Comparative study of comprehensive gas chromatography-nitrogen chemiluminescence detection and gas chromatography-ion trap-tandem mass spectrometry for determining nicotine and carcinogen organic nitrogen compounds in thirdhand tobacco smoke, *J. Chromatogr. A* 1426 (2015) 191–200.
- [200] N. Ramirez, M.Z. Ozel, A.C. Lewis, R.M. Marce, F. Borrull, J.F. Hamilton, Determination of nicotine and N-nitrosamines in house dust by pressurized liquid extraction and comprehensive gas chromatography-nitrogen chemiluminescence detection, *J. Chromatogr. A* 1219 (2012) 180–187.
- [201] A.K. Venkatesan, B.F. Pycke, R.U. Halden, Detection and occurrence of N-nitrosamines in archived biosolids from the targeted national sewage sludge survey of the U.S. Environmental Protection Agency, *Environ. Sci. Technol.* 48 (2014) 5085–5092.
- [202] A.J. Gushgari, R.U. Halden, A.K. Venkatesan, Occurrence of N-nitrosamines in U.S. Freshwater sediments near wastewater treatment plants, *J. Hazard. Mater.* 323 (2017) 109–115.
- [203] J.E. Seo, J.E. Park, J.Y. Lee, H. Kwon, Determination of seven N-nitrosamines in agricultural food matrices using GC-PCI-MS/MS, *Food Anal. Methods* 9 (2016) 1595–1605.
- [204] Q. Zhu, J. Wang, S. Liu, Y. Zhang, Determination of four volatile n-nitrosamines in the process of Chinese preserved meat, *International Conference on Materials, Environmental and Biological Engineering* (2015) 618–621.
- [205] G. Drabik-Markiewicz, B. Dejaegher, E. De Mey, T. Kowalska, H. Paelinck, Y. Vander Heyden, Influence of putrescine, cadaverine, spermidine or spermine on the formation of N-nitrosamine in heated cured pork meat, *Food Chem.* 126 (2011) 1539–1545.
- [206] L. Li, P. Wang, X. Xu, G. Zhou, Influence of various cooking methods on the concentrations of volatile N-nitrosamines and biogenic amines in dry-cured sausages, *J. Food Sci.* 77 (2012) C560–C565.
- [207] N.V. Komarova, A.A. Velikanov, Determination of volatile N-nitrosamines in food by high-performance liquid chromatography with fluorescence detection, *J. Anal. Chem.* 56 (2001) 359–363.
- [208] F. Wei, X. Xu, G. Zhou, G. Zhao, C. Li, Y. Zhang, L. Chen, J. Qi, Irradiated Chinese Rugao ham: changes in volatile N-nitrosamine, biogenic amine and residual nitrite during ripening and post-ripening, *Meat Sci.* 81 (2009) 451–455.
- [209] N.P. Sen, S. Seaman, W.F. Miles, Volatile nitrosamines in various cured meat-products - effect of cooking and recent trends, *J. Agric. Food Chem.* 27 (1979) 1354–1357.
- [210] T.A. Gough, K. Goodhead, C.L. Walters, Distribution of some volatile nitrosamines in cooked bacon, *J. Sci. Food Agric.* 27 (1976) 181–185.
- [211] G.M. Telling, T.A. Bryce, J. Althorpe, Use of vacuum distillation and gas chromatography-mass spectrometry for determination of low levels of volatile nitrosamines in meat products, *J. Agric. Food Chem.* 19 (1971), 937–&.
- [212] L. Ceh, F. Ender, A sensitive method for the colorimetric determination of volatile nitrosamines in food products and air, *Food Cosmet. Toxicol.* 16 (1978) 117–121.
- [213] ASTM, Standard Specification for Volatile N-Nitrosamine Levels in Rubber Nipples on Pacifiers, in: ASTM International, ASTM F1313-90(2011), 2011.
- [214] European Committee for Standardization, Child use and care articles. Method for determining the release of N-nitrosamines and N-nitrosatable substances from elastomer or rubber teats and soothers, in: EN 12868, 2017, 2017.
- [215] B. Jurado-Sanchez, E. Ballesteros, M. Gallego, Comparison of microwave assisted, ultrasonic assisted and Soxhlet extractions of N-nitrosamines and aromatic amines in sewage sludge, soils and sediments, *Sci. Total Environ.* 463–464 (2013) 293–301.

- [216] W. Fiddler, J.W. Pensabene, Supercritical fluid extraction of volatile N-nitrosamines in fried bacon and its drippings: method comparison, *J. AOAC Int.* 79 (1996) 895–901.
- [217] H. Kodamatani, S.L. Roback, M.H. Plumlee, K.P. Ishida, H. Masunaga, N. Maruyama, T. Fujioka, An inline ion-exchange system in a chemiluminescence-based analyzer for direct analysis of N-nitrosamines in treated wastewater, *J. Chromatogr. A* 1553 (2018) 51–56.
- [218] German Federal Institute for Drugs and Medical Devices, Ausweitung Des Verfahrens Auf Weitere Sartane, 2018, [https://www.bfarm.de/SharedDocs/Risikoinformationen/Pharmakovigilanz/DE/RV\\_STP/s-z/valsartan.html](https://www.bfarm.de/SharedDocs/Risikoinformationen/Pharmakovigilanz/DE/RV_STP/s-z/valsartan.html).
- [219] X. Wang, H. Yang, B. Zhou, X. Wang, Y. Xie, Effect of oxidation on amine-based pharmaceutical degradation and N-Nitrosodimethylamine formation, *Water Res.* 87 (2015) 403–411.
- [220] D. Hanigan, I. Ferrer, E.M. Thurman, P. Herckes, P. Westerhoff, LC/QTOF-MS fragmentation of N-nitrosodimethylamine precursors in drinking water supplies is predictable and aids their identification, *J. Hazard. Mater.* 323 (2017) 18–25.
- [221] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH M7(R1) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, 2017.