

# SIMPLE, RAPID ANALYSIS OF N-NITROSODIMETHYLAMINE (NDMA) IMPURITY IN RANITIDINE PRODUCTS USING SIFT-MS

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Quantitative analysis of volatile nitrosamine impurities in drug products is greatly simplified using SIFT-MS and has a three-fold throughput advantage (excluding sample prep benefits) over chromatographic methods.

## Abstract

Mutagenic N-nitrosamine impurities are found at trace concentrations in certain pharmaceutical products as byproducts of synthesis or, less commonly, through migration from packaging materials. They are traditionally analyzed using chromatographic techniques that require significant sample preparation and have relatively low sample throughputs. SIFT-MS simplifies and speeds up analysis of trace volatile N-nitrosamine impurities, with a throughput of 12 samples/hr (three times faster than gas and liquid chromatography methods assuming they utilize fully automated sample preparation) and only 70 minutes to first result (including full calibration set; over twice as fast as chromatographic methods). This application note describes headspace-SIFT-MS analysis of ranitidine products and achieves a limit of quantitation of 2 ng g<sup>-1</sup> for NDMA in 500 mg of drug product.

## INTRODUCTION

The known or suspected mutagenicity of many N-nitrosamines – in particular, N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) – means that their presence in any product to which humans are exposed is of concern. Despite the occurrence of these compounds being well-known in water, beverages, and foods, their discovery in sartan medicines in 2018 came as something of a shock to the pharmaceutical industry (Golob et al. (2022) and references therein). Elastomeric sources of N-nitrosamines – as used in various packaging components – are typically very well controlled due to improvements made in the manufacture of elastomers due to historic issues in that industry (Boltres (2021)). Investigations have revealed that most N-nitrosamine issues arise from nitrosating agents used in synthesis – especially when secondary amines are present (EMA (2021); US FDA (2021)).

Following substantial industry investigation and consultation, the European Medicines Agency (EMA) and United States Food and Drug Administration (FDA) have issued acceptable intakes (EMA (2021); US FDA (2021)). The limits for the more volatile N-nitrosamines are summarized in Table 1. With acceptable intakes at low nanogram levels per day, highly sensitive and

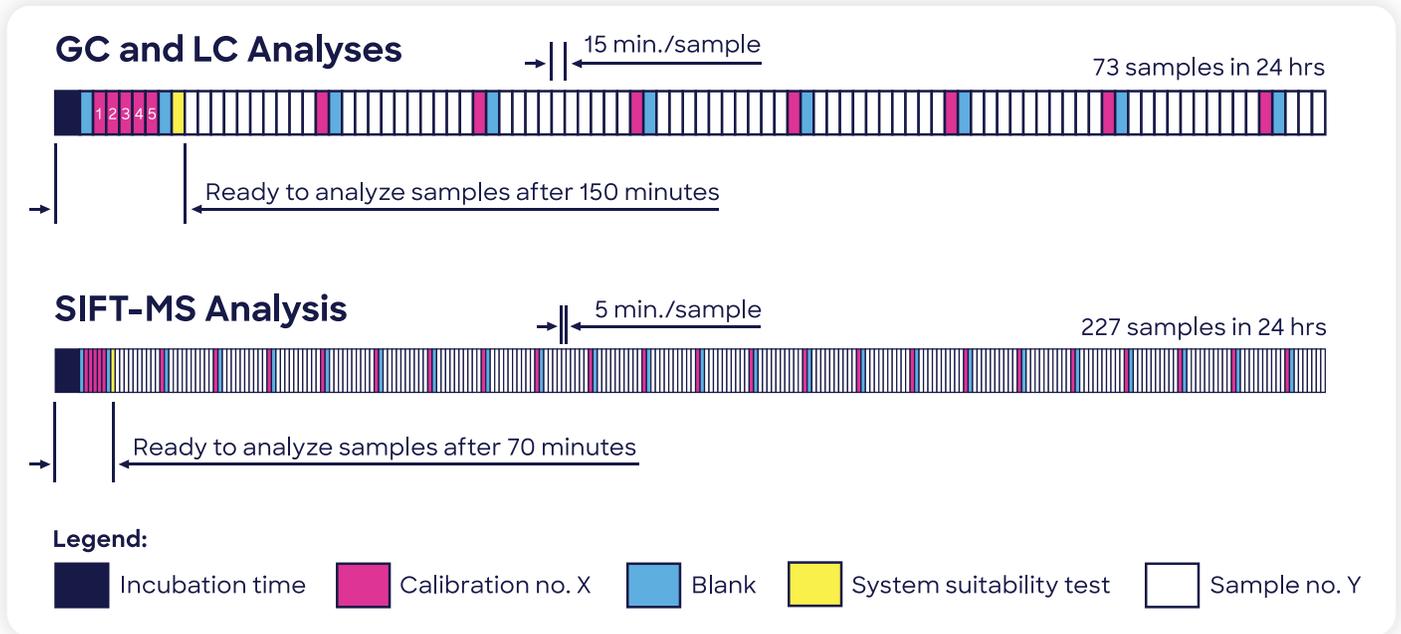
selective analytical methods are required. Typically, these are based on gas or liquid chromatography with longer analysis times and more complex sample preparation (e.g., dissolution and centrifugation needed). They are also off-line methods, having a relatively long time to result for the first sample when calibration is considered, and a relatively low sample throughput. In contrast, selected ion flow tube mass spectrometry (SIFT-MS) greatly simplifies sample preparation (drug product is simply weighed into the vial) and eliminates the slow chromatography step, by utilizing soft chemical ionization directly to gas-phase and headspace samples. This approach not only speeds up the analysis, but it can also significantly reduce other sample preparation because no derivatization of the highly polar nitrosamines is required (Langford et al. (2015)).

This application note demonstrates that headspace-SIFT-MS is well suited to quantitative screening analysis of volatile nitrosamines in drug products because it is highly sensitive and selective. It also provides significant advantages over routine chromatographic analysis through delivery of faster results and higher sample throughputs (Figure 1).

**Table 1.** Volatile nitrosamines of concern to the United States FDA and EMA, with acceptable intakes for each regulatory agency.

Compounds (Acronym)	CAS No.	MW / g mol <sup>-1</sup>	FDA acceptable intake / ng day <sup>-1</sup>	EMA acceptable intake / ng day <sup>-1</sup>
N-nitrosodimethylamine (NDMA)	62-75-8	74.08	96	96
N-nitrosodiethylamine (NDEA)	55-18-5	102.14	26.5	26.5
N-nitrosoethylisopropylamine (NEIPA)	16339-04-1	116.16	26.5	26.5
N-nitrosodiisopropylamine (NDIPA)	601-77-4	130.19	26.5	26.5
N-nitrosodipropylamine (NDPA)	621-64-7	130.19		26.5
1-methyl-4-nitrosopiperazine (MeNP)	16339-07-4	129.16		26.5
N-nitrosopiperidine	100-75-4	114.15		1300
N-nitrosodibutylamine (NDBA)	924-16-3	158.24		26.5

Figure 1. Headspace SIFT-MS enables high-throughput screening of volatile nitrosamines, providing enhanced quality control and quality assurance.



## Method

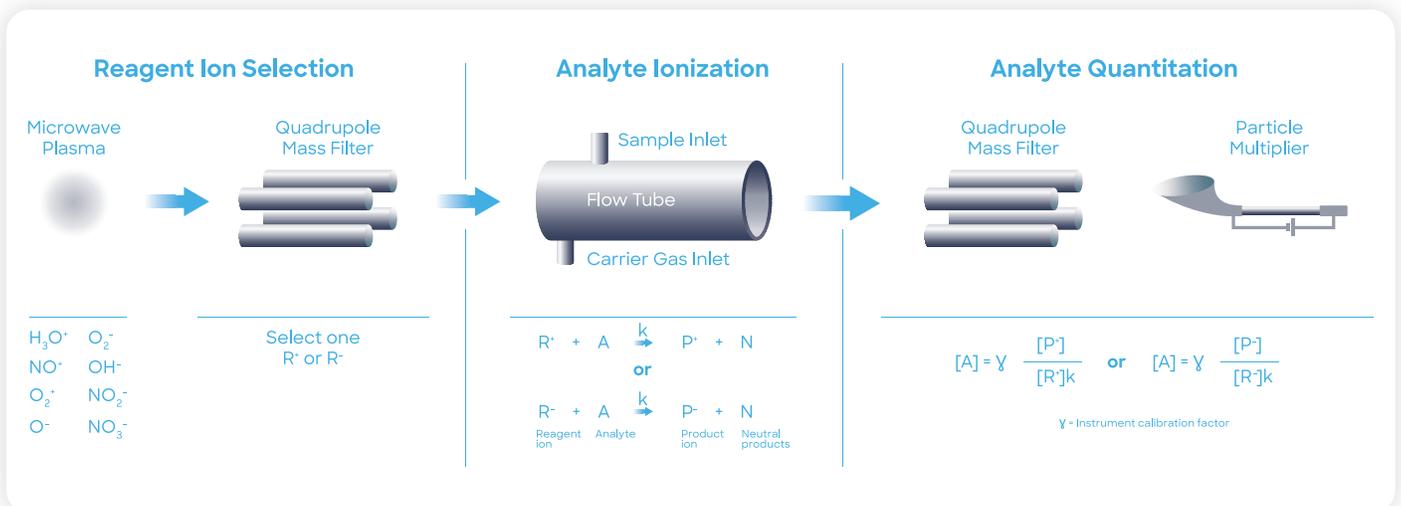
### 1. The SIFT-MS technique

This work utilized a Syft Technologies Voice200ultra SIFT-MS instrument operating on helium carrier gas. SIFT-MS (Figure 2) uses soft chemical ionization (CI) to generate mass-selected reagent ions (Smith et al. (2020)) that can rapidly react with and quantify volatile organic compounds (VOCs) down to part-per-trillion concentrations (by volume, pptV). Up to eight reagent ions ( $\text{H}_3\text{O}^+$ ,  $\text{NO}^+$ ,  $\text{O}_2^+$ ,  $\text{O}^+$ ,  $\text{OH}^+$ ,  $\text{O}_2^-$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$ ) obtained from a microwave discharge in air are now applied in commercial SIFT-MS instruments (Hera et al. (2017)). These reagent ions react with VOCs and other trace analytes in well-controlled ion-molecule reactions, but they do not react with the major components of air ( $\text{N}_2$ ,  $\text{O}_2$  and Ar). This enables direct, real-time analysis of air samples to be achieved at trace and ultra-trace levels without pre-concentration. Rapid switching between reagent ions provides high selectivity because the multiple reaction mechanisms give independent

measurements of each analyte. The multiple reagent ions frequently remove uncertainty from isobaric overlaps in mixtures containing multiple analytes.

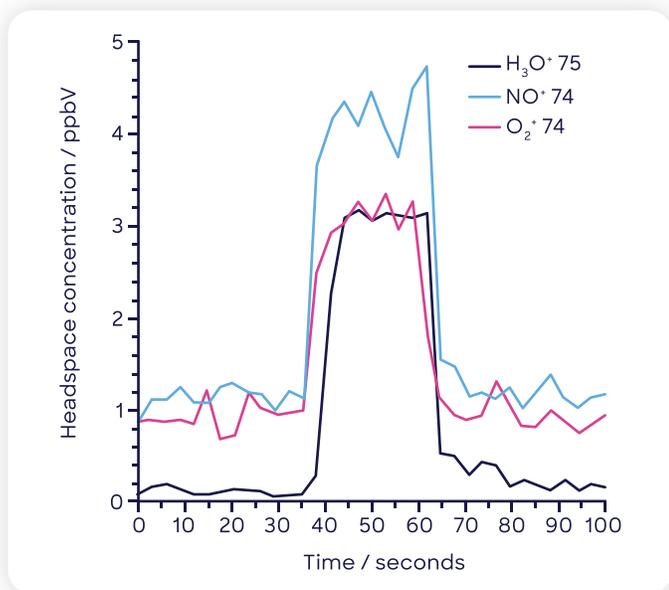
Automated MHE analysis was carried out using a SIFT-MS instrument coupled with a multipurpose autosampler (MPS Robotic Pro, GERSTEL; Mülheim, Germany). The autosampler was controlled using GERSTEL's Maestro software. Samples were incubated at 60 °C for 30 min. in a GERSTEL agitator. Headspace was sampled using a 2.5-mL headspace syringe (heated to 150 °C) and subsequently injected at a flow rate of 100  $\mu\text{L s}^{-1}$  into the SIFT-MS instrument's autosampler inlet (heated to 150 °C) via a self-sealing GERSTEL septumless sampling head. Since the nominal sample flow into the SIFT-MS instrument is 370  $\mu\text{L s}^{-1}$ , a make-up gas flow (ultra-high purity nitrogen) is also introduced through the sampling head. This dilution is accounted for in the calibration curve which was generated under the same conditions as the samples were measured. The analysis time for each sample

Figure 2. Schematic diagram of SIFT-MS - a direct, chemical-ionization analytical technique.



was 100 s (Figure 3) and reported concentrations are the mean of the values obtained during injection (i.e., between ca. 40 and 60 s). Note that no internal standard was utilized (Perkins and Langford (2021a)).

**Figure 3.** Example headspace injection with synchronous SIFT-MS analysis of NDMA at 2.6-ng spike level (10-mL sample vial).



## 2. SIFT-MS analysis of NDMA and matrix effects

SIFT-MS analysis of a variety of volatile nitrosamines has been described previously (Langford et al. (2015)). Table 2 summarizes the reaction chemistry for NDMA, the target analyte in this study. Reaction rate coefficients,  $k$ , are the primary measure of SIFT-MS sensitivity and are higher for nitrosamines than for most VOCs due to their high polarity. Selectivity is achieved due to the availability of multiple product ions per compound, which enables cross comparison of independent concentration measurements from the individual product ions. N,N-Dimethylformamide (DMF) is included because it is the most probable interferent for NDMA through its  $^{13}\text{C}$  isotope peak. This interference, when it occurs, is easily remedied through subtraction from the

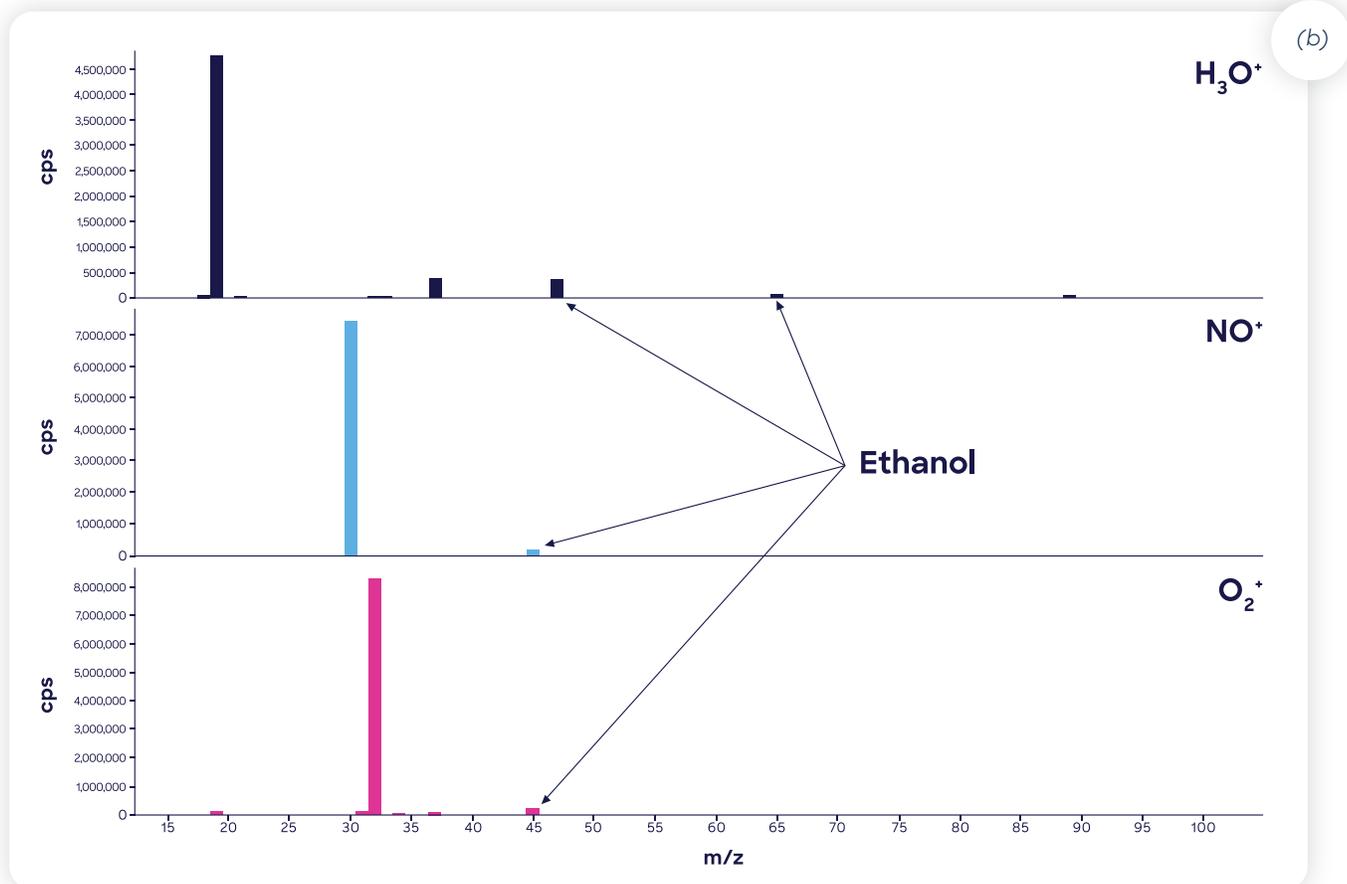
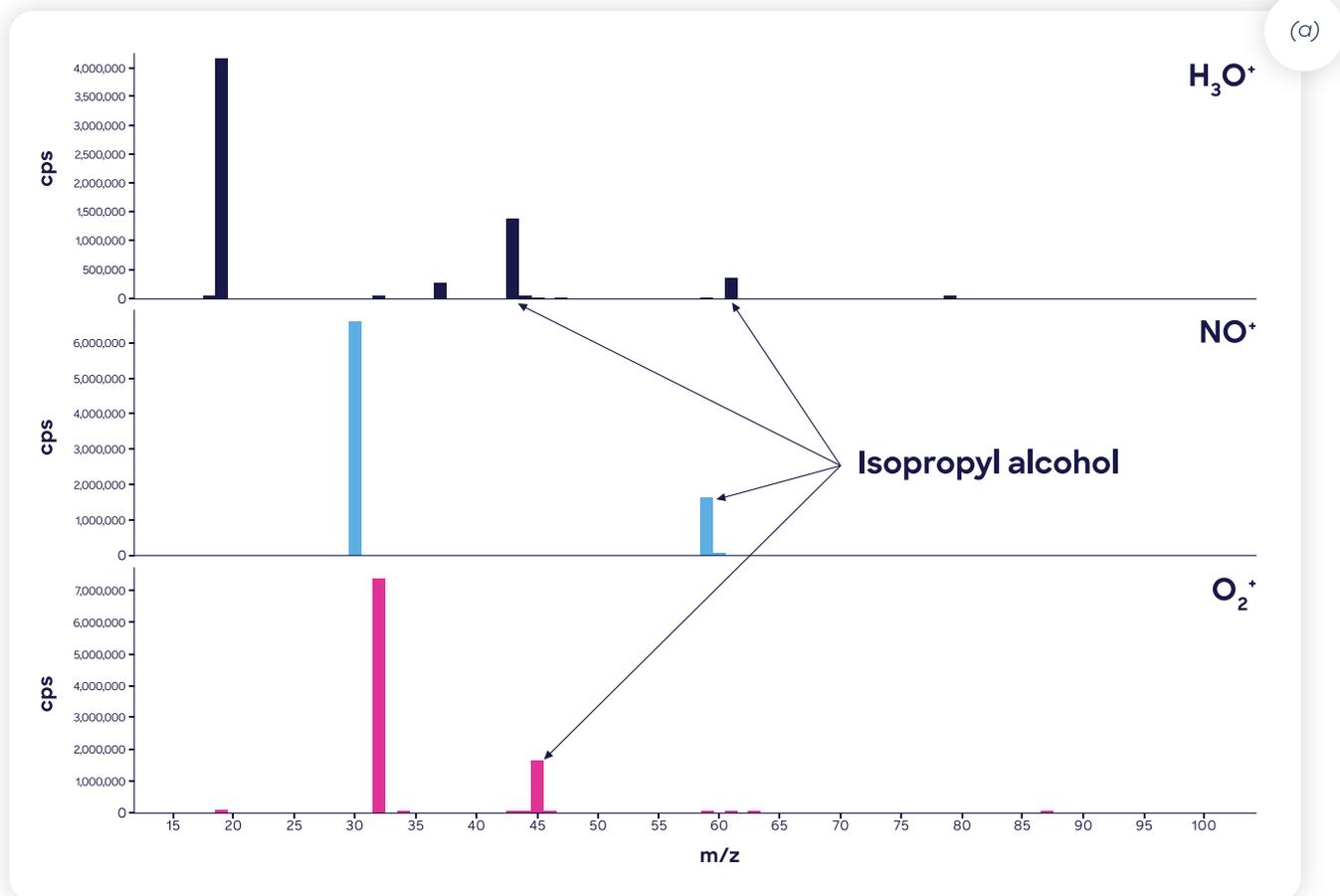
**Table 2.** SIFT-MS reaction chemistry (rate coefficient ( $k$ ), product ion formulae, branching ratio (BR as %), and mass-to-charge ratio ( $m/z$ ) for NDMA and N,N-dimethylformamide (DMF), a potential interferent. Ions in gray are not ordinarily used for quantitation or identification due to their low BR (reduced sensitivity).

Reagent Ion	N-Nitrosodimethylamine (NDMA)				N,N-Dimethylformamide (DMF)			
	$k$	Formula	BR	$m/z$	$k$	Formula	BR	$m/z$
$\text{H}_3\text{O}^+$	$4.9 \times 10^{-9}$	$(\text{CH}_3)_2\text{N}_2\text{O.H}^+$	100%	75	$3.3 \times 10^{-9}$	$\text{C}_3\text{H}_7\text{NO.H}^+$ CHO <sup>+</sup>	95% 5%	74 29
$\text{NO}^+$	$3.8 \times 10^{-9}$	$(\text{CH}_3)_2\text{N}_2\text{O}^+$	100%	74	$2.5 \times 10^{-9}$	$\text{C}_3\text{H}_7\text{NO}^+$ $\text{C}_3\text{H}_6\text{NO}^+$ $\text{C}_3\text{H}_7\text{NO.NO}^+$	45% 45% 10%	73 72 103
$\text{O}_2^+$	$4.1 \times 10^{-9}$	$(\text{CH}_3)_2\text{N}_2\text{O}^+$ $(\text{CH}_3)_2\text{N}_2\text{O}^+$	95% 95%	74 44	$2.9 \times 10^{-9}$	$\text{C}_3\text{H}_7\text{NO}^+$ $\text{C}_3\text{H}_7^+$	95% 5%	73 43

apparent NDMA signal. This correction has been applied in the concentration data presented in this application note.

In addition to potential interference by DMF, high levels of residual solvent impact calculation of concentrations in SIFT-MS due to excessive consumption of reagent ions. High concentrations are evident in the non-linear responses to drug product mass for  $\text{H}_3\text{O}^+$  and  $\text{O}_2^+$ ; this phenomenon needs to be understood and mitigated in method development. Here, the  $\text{NO}^+$  reagent is often more robust to this effect than are  $\text{H}_3\text{O}^+$  and  $\text{O}_2^+$  due to it being somewhat less sensitive to very common solvents such as ethanol or isopropyl alcohol. Figure 4 shows a full scan analysis of one of the drug products with spectral features arising from these solvents annotated.

Figure 4. SIFT-MS full-scan mass spectra of the two ranitidine-containing drug products containing relatively high levels of residual solvents (a) isopropyl alcohol in Product 1 and (b) ethanol in Product 2. The largest spectral features peaks are from the reagent ions,  $H_3O^+$  ( $m/z$  19),  $NO^+$  (30), and  $O_2^+$  (32).



### 3. Drug product samples

The focus of this application note is method development, rather than testing of a large number of drug samples. Here, two commercial ranitidine products were analyzed for NDMA content. Powdered samples (100 to 500 mg) were placed in 10 mL headspace vials for headspace analysis without subsequent dissolution in water (i.e., of the powder itself), which is termed “direct analysis” here. This enabled a reduction in size of headspace vial from the ‘standard’ recommended volume of 20 mL (Perkins and Langford (2021b)) to increase sensitivity.

## Results and Discussion

Two studies are presented here: First, quantitation of NDMA in the gas phase using headspace-SIFT-MS is evaluated. Second, quantitative analysis of NDMA in ranitidine products is demonstrated.

### 1. NDMA detection linearity and LOQ

The first step in method development was to confirm linear detection of NDMA and obtain an estimated LOQ for headspace analysis. The two sets of data in Figure 5 were generated by serial dilution of fully evaporated NDMA solutions. The higher range on the left was generated from full evaporation of 1  $\mu\text{L}$  of commercial NDMA standard (5 mg mL<sup>-1</sup> in methanol). A 1- $\mu\text{L}$  aliquot of the headspace was diluted in a nitrogen-filled 20-mL sample vial. From this, 250, 500, 750, 1000, and 1250  $\mu\text{L}$  aliquots were placed in five vials, giving the masses of NDMA shown on the horizontal x axis. The lower range (Figure 5(b)) involved pre-dilution of the commercial methanolic standard in 50% acetone prior to creation of the vapor-phase stock via a 0.5- $\mu\text{L}$  spike into a 20-mL headspace vial – hence the four-fold dilution, and then creation of the various levels.

All three ions targeted in the analysis of NDMA are shown because they illustrate the second matrix effect described in the Methods section: the presence of solvents (methanol and methanol plus acetone). Note that acetone was used as a diluent in an attempt to provoke the same non-linear response in NO<sup>+</sup>, since acetone reacts significantly faster with this reagent ion than does methanol, but the NO<sup>+</sup> remained robust. Although the solvents do not originate from a drug product, but from the calibration mixture, the effect is the same. With no chromatographic separation, they are present in the ionization region of the SIFT-MS instrument (the flow tube) and compete with NDMA for the selected SIFT-MS reagent ion. At the high concentration end of Figure 5(a) there is ca. 60 ppmV of methanol in the headspace. NO<sup>+</sup> is unaffected by methanol, but H<sub>3</sub>O<sup>+</sup> (especially) and O<sub>2</sub><sup>+</sup> are, causing increasing over-reporting as the concentration increases. Because of this behavior, the NO<sup>+</sup> product ion (m/z 74) is used as the quantitation ion, while O<sub>2</sub><sup>+</sup> and H<sub>3</sub>O<sup>+</sup> are used as qualifier ions. Figure 6 shows the combined range for the quantitation ion.

Figure 5. NDMA response in presence of (a) methanol and (b) 50:50 methanol:acetone mix. See the text for details on how these curves were created.

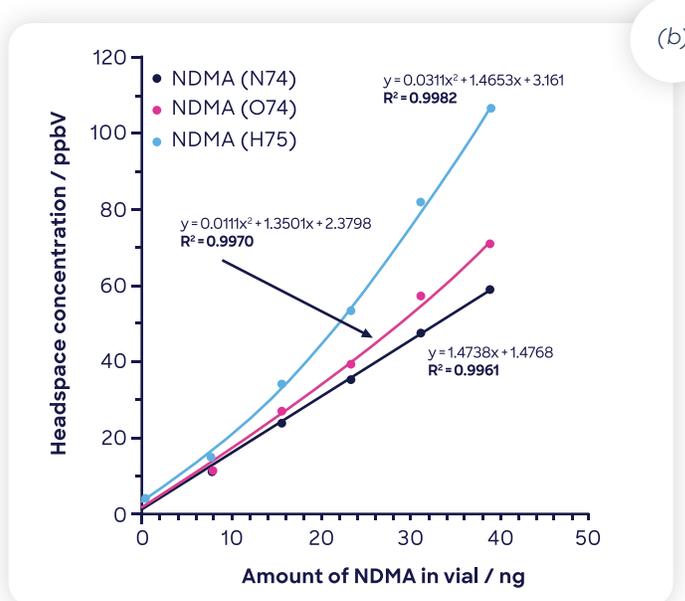
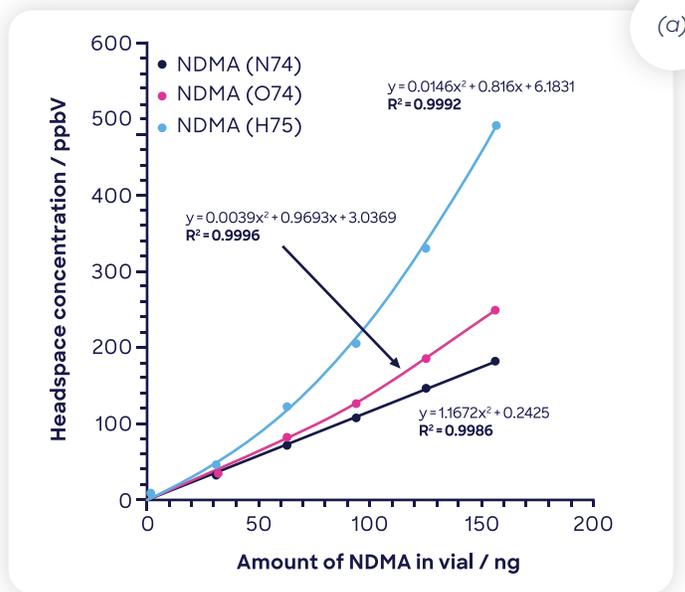
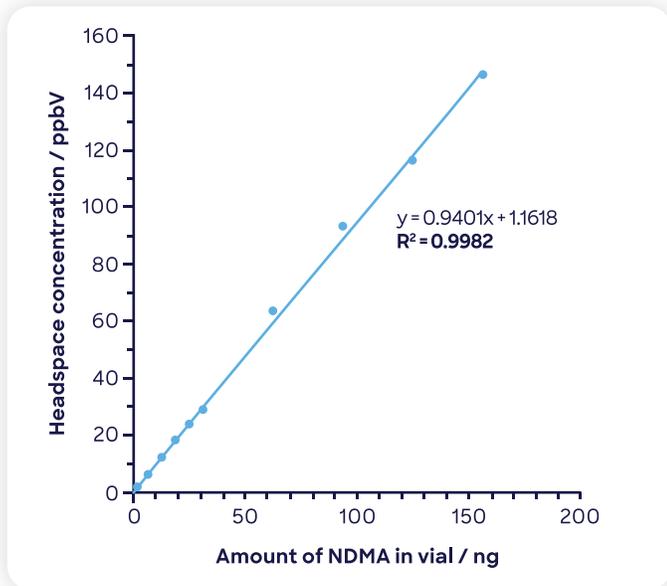


Figure 6. NDMA across full range showing just the quantitation ion, NO<sup>+</sup> m/z 74.



The signal-to-noise ratio (S/N), limit of detection (LOD), and limit of quantitation (LOQ) for gas-phase SIFT-MS analysis of NDMA are summarized in Table 3. These are specific to the use of a 2.5-mL aliquot of headspace injected at 100  $\mu\text{L s}^{-1}$  and were calculated from the low-concentration headspace injection shown in Figure 3. From these data the LOQ for drug products is estimated at 2 ng  $\text{g}^{-1}$  for a 500 mg sample.

Table 3. Signal-to-noise (S/N), limit of detection (LOD), and limit of quantitation (LOQ) for gas-phase SIFT-MS analysis of NDMA in a 2.5-mL aliquot injected at 100  $\mu\text{L s}^{-1}$ .

NDMA Reagent Ion - Product Ion Pair	Signal-to-Noise Ratio (S/N)	Theoretical LOD based on 3:1 S/N / ng	Theoretical LOQ based on 10:1 S/N / ng
H <sub>2</sub> O <sup>+</sup> 75	23.8	0.33	1.1
NO <sup>+</sup> 74	15.3	0.51	1.7
O <sub>2</sub> <sup>+</sup> 74	5.9	1.3	4.4

## 2. Quantitative analysis of NDMA in solid drug product

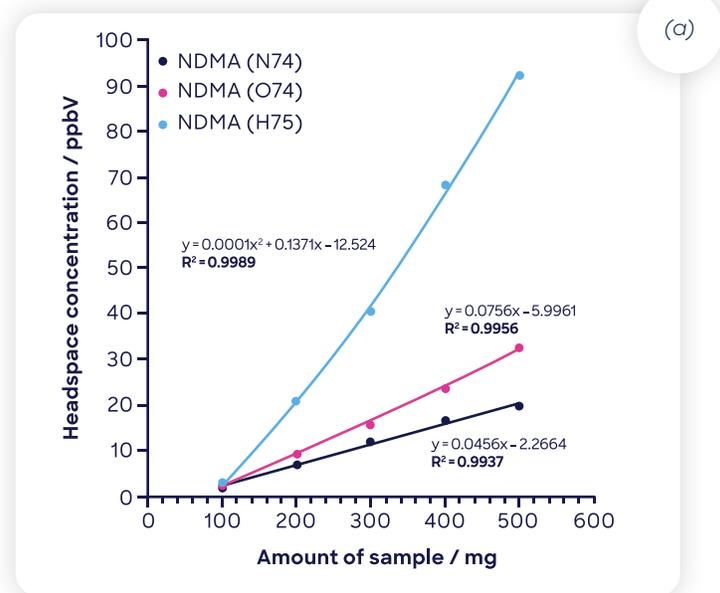
Multiple headspace extraction (MHE) enables absolute concentrations to be determined in condensed phases independent of the matrix (Perkins and Langford (2022) and references therein). Hence it is a powerful technique for direct quantitative analysis of solid dose forms because it avoids complicated matrix matching procedures. Due to faster sample analysis, the SIFT-MS technique makes this repeat-analysis methodology significantly more economical, though the end goal is not to do full MHE on all samples; but correlate the first injection with full MHE and apply a correction factor (Perkins and Langford (2022)).

Successful MHE requires analysis over a dynamic range of ca. two orders of magnitude, so the first step is to optimize the amount of sample that should be utilized from static headspace analysis (at equilibrium; see Figure 7). For NO<sup>+</sup>, signal levels suggest that 500 mg of drug product can be utilized. Hence full MHE was conducted, with the results obtained over six injections plotted against the natural logarithm of concentration in Figure 8. It is evident that Product 1 (Figure 8(a)) has no ions that behave linearly with injection number, while NO<sup>+</sup> and O<sub>2</sub><sup>+</sup> behave well for

Product 2. However, under the incubation conditions used, the NDMA concentration in both drug products is essentially at the baseline by the sixth injection. This means that even when the conventional extrapolation approach to determining total concentration in the drug product (Perkins and Langford (2022)) cannot be adopted, the individual concentration measurements can be summed. For Product 2 the conventional area-under-the-curve-approach was used to derive the final concentration, while for Product 1 the values obtained for injections 1 – 6 were added together. The NDMA concentrations in Products 1 and 2 are 68 and 328 ng  $\text{g}^{-1}$ , respectively. These are in the range reported to the US FDA for ranitidine products (US FDA (2019)). Full MHE-SIFT-MS provides a sample throughput of 40 samples per day with a 30-min. incubation (see Figure 9 in Perkins and Langford (2022)), whereas MHE-GC will analyze only 5 samples per day.

Despite some of the MHE curves not behaving linearly, the results obtained are very repeatable as triplicate measurements obtained for 300 mg samples demonstrate (Figure 9). These data used the full six-injection MHE approach, and no internal standard was used, as is common practice with SIFT-MS. This illustrates the high repeatability of the SIFT-MS technique even in non-ideal matrices.

Figure 7. Measured headspace concentration of NDMA using SIFT-MS as a function of sample mass for two ranitidine drug products.



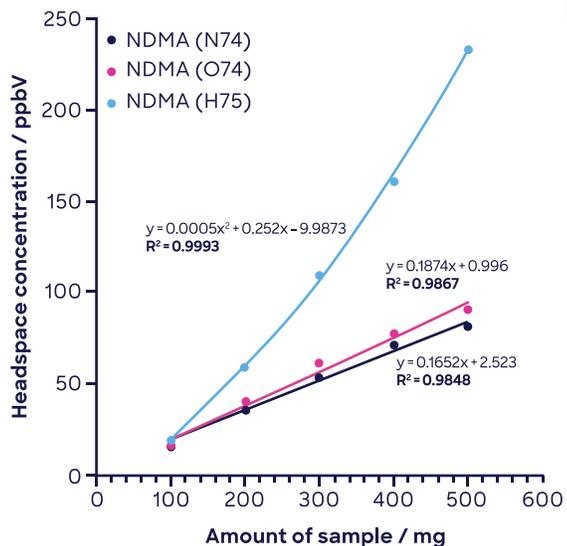
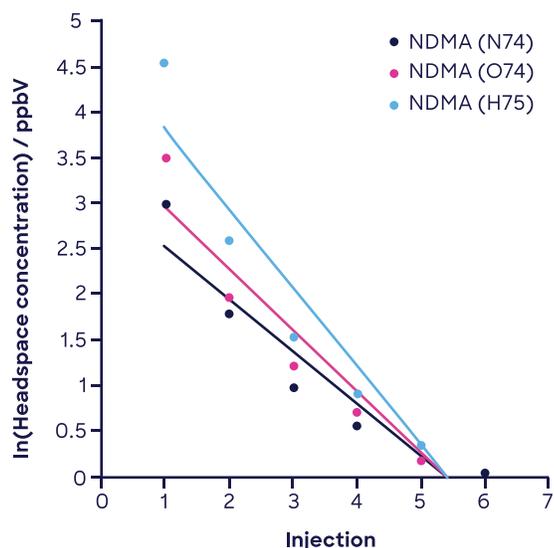
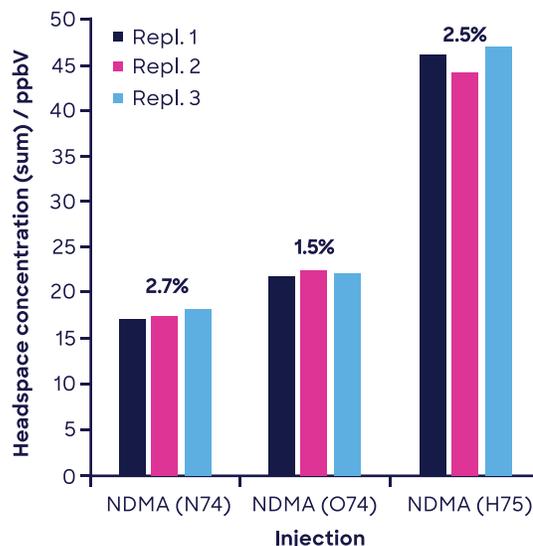


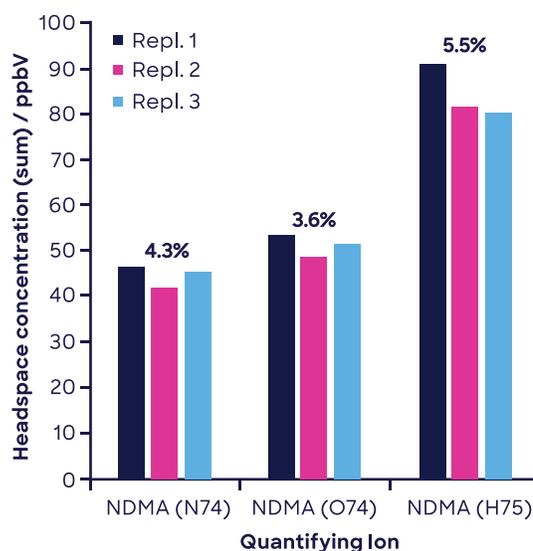
Figure 8. MHE-SIFT-MS results (natural logarithm of headspace concentration as function of injection number) for NDMA in 500-mg samples of two ranitidine drug products.



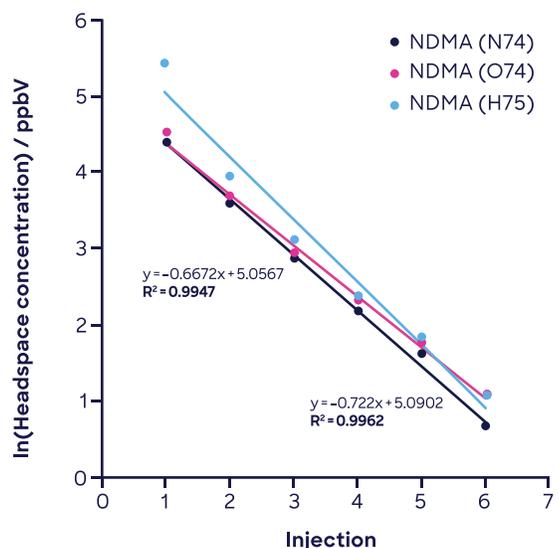
(a)



(a)



(b)

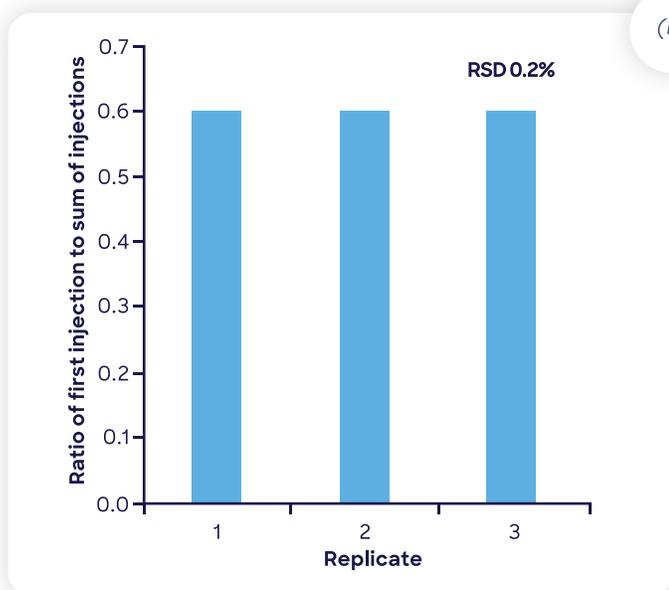
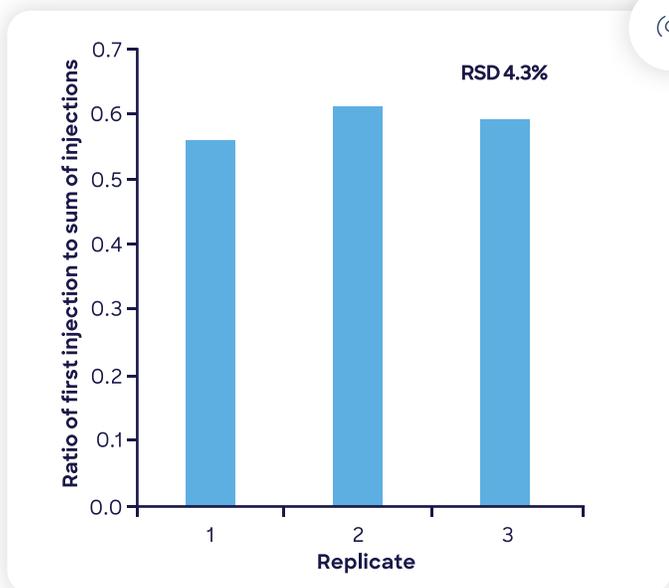


(b)

Figure 9. Repeatability of triplicate MHE-SIFT-MS measurements of NDMA (using  $\text{NO}^+ 74$ ) in 300-mg samples of the two ranitidine drug products.

The excellent repeatability enables use of a single headspace injection per sample rather than full MHE because the latter is repeatably correlated to the first injection as demonstrated for MHE-SIFT-MS analysis of polystyrene (Perkins and Langford (2022)). Note the sample must be at equilibrium. Figure 10 shows the correlation between the first injection and the sum of injections (1 to 6) for the repeatability data given in Figure 9. The replicates demonstrate excellent repeatability, with close to 0.6 of the total NDMA in the sample partitioning to the headspace in the first 30-minute incubation cycle at 60 °C. This result means that there is potential to screen 250+ samples/day for very low nanogram-per-gram quantities of NDMA using headspace-SIFT-MS. This is ca. four-fold higher throughput than can be achieved using GC and LC with automated sample preparation, or over six-fold higher for manual sample preparation.

Figure 10. Repeatability of the ratio of first injection to the sum of all injections for the two ranitidine drug products shown in Figure 9.



## CONCLUSIONS

- High-sensitivity headspace-SIFT-MS analysis detects NDMA in the required very low  $\text{ng g}^{-1}$  range.
- Headspace-SIFT-MS provides significantly faster time to result and higher throughputs than conventional chromatographic methods.
- Quantitative analysis direct from solid / powder from a single incubation cycle is possible through correlation with MHE-SIFT-MS.
- Simple sample preparation: powder in vial and go! (No derivatization, preconcentration, etc.)
- Simple operation.
- Industry-proven technology ready for the QA/QC lab and process line.

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