# Recent advances and challenges in the analysis of mutagenic impurities in pharmaceutical products

## Pharmaceutical Quality Regulatory Science
## November 20

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# Outline

1. Introduction
2. Analytical methods for genotoxic impurity (GTI)
3. Examples of analyzing GTIs in drug development
4. Challenges in analysis of GTIs
5. Conclusion

# Generation of genotoxic impurities/degradants

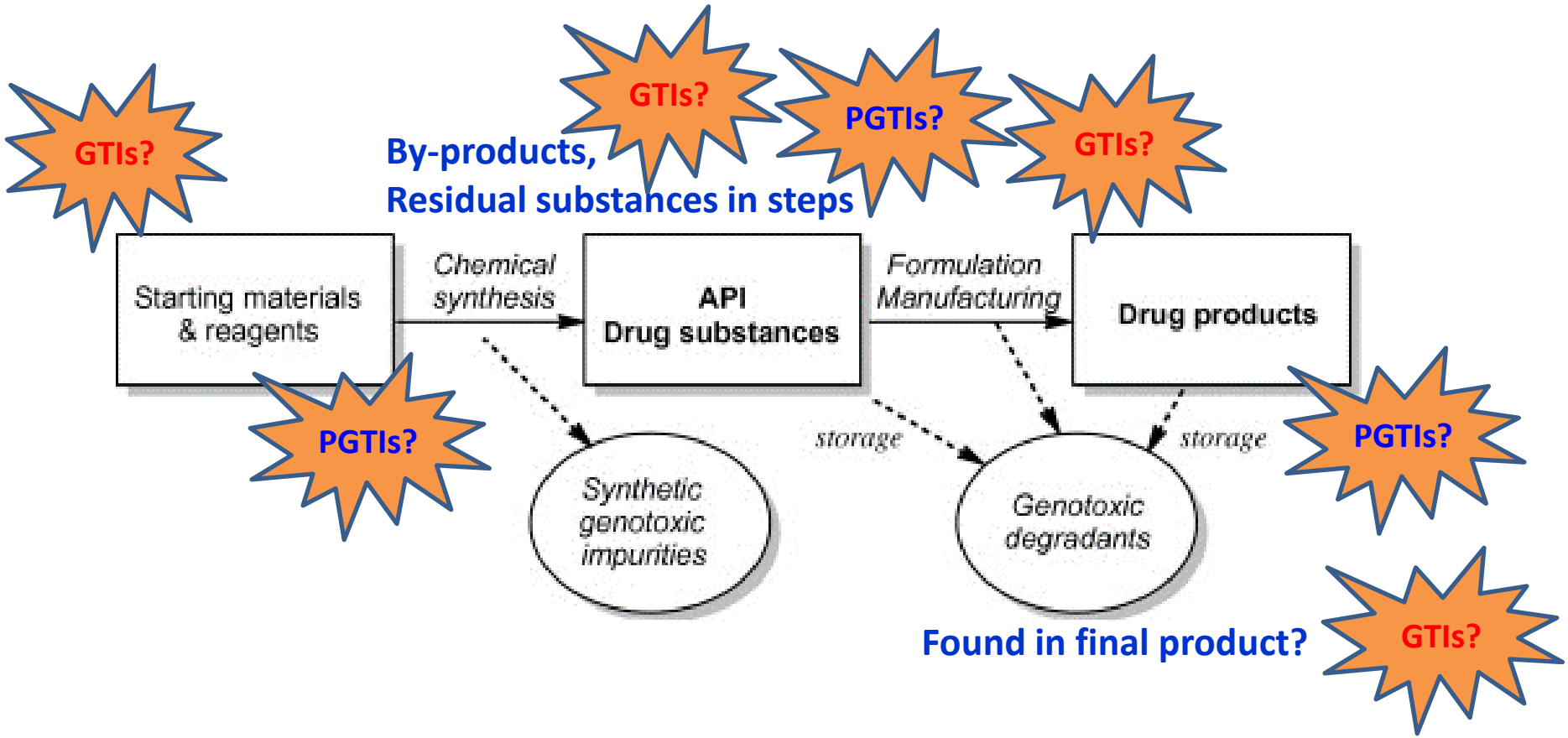
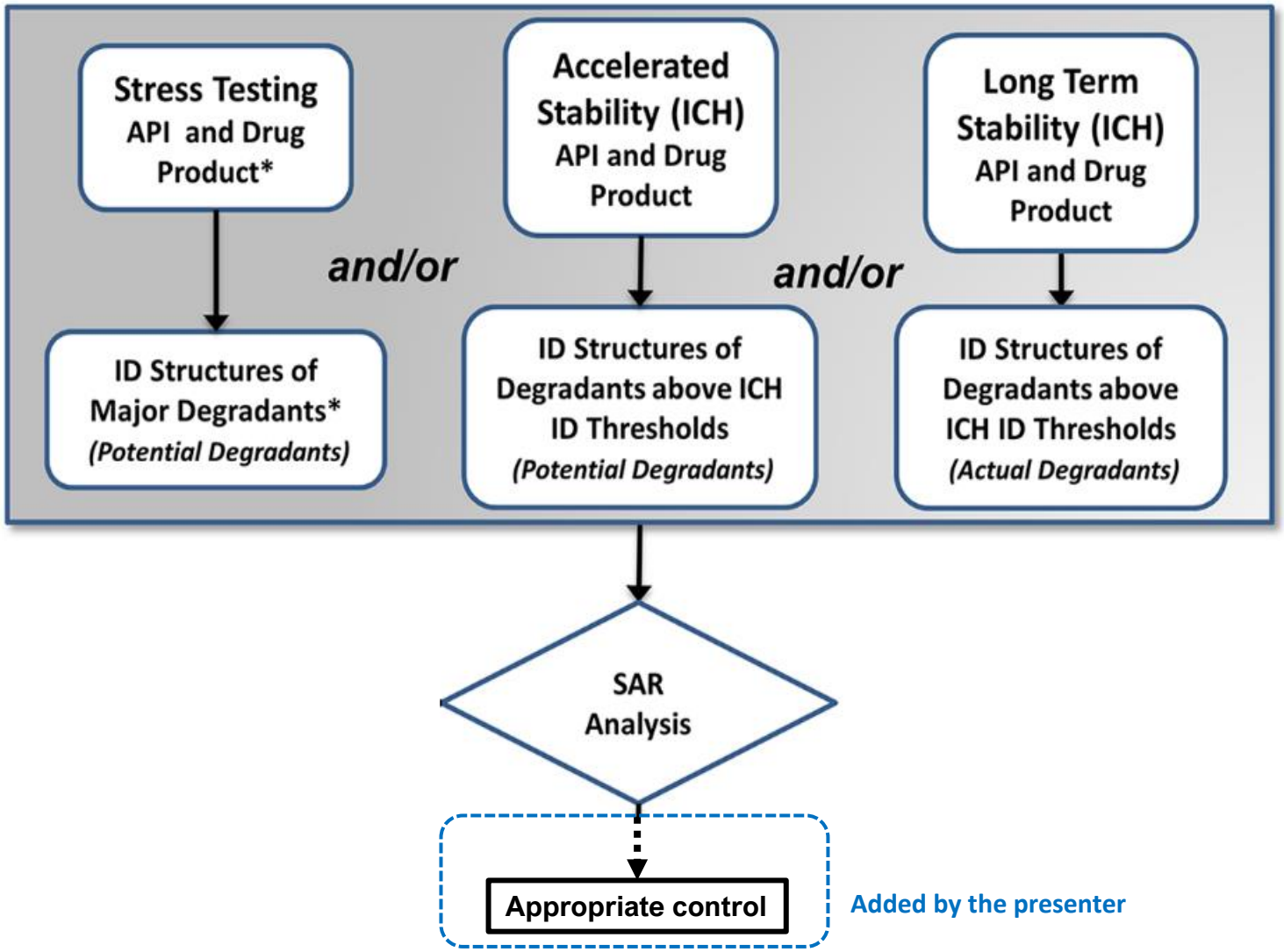



**Fig. A typical chemistry and formulation process showing where synthetic genotoxic impurities and genotoxic degradants can be generated.**

TrAC Trends in Analytical Chemistry Volume 49, September 2013, Pages 108-117

# Process Flow for Assessing Degradants in Drug Substance and Drug Product




Mark H. Kleinman et al., Organic Process Research & Development 19 (2015) 1447-1457.

# Outline

1. Introduction
2. Analytical methods for genotoxic impurity (GTI)
    1. Strategy of the method development
    2. NDMA analysis
3. Examples of analyzing GTIs in drug development
4. Challenges in analysis of GTIs
5. Conclusion

# Evaluation of PGTIs and GTIs

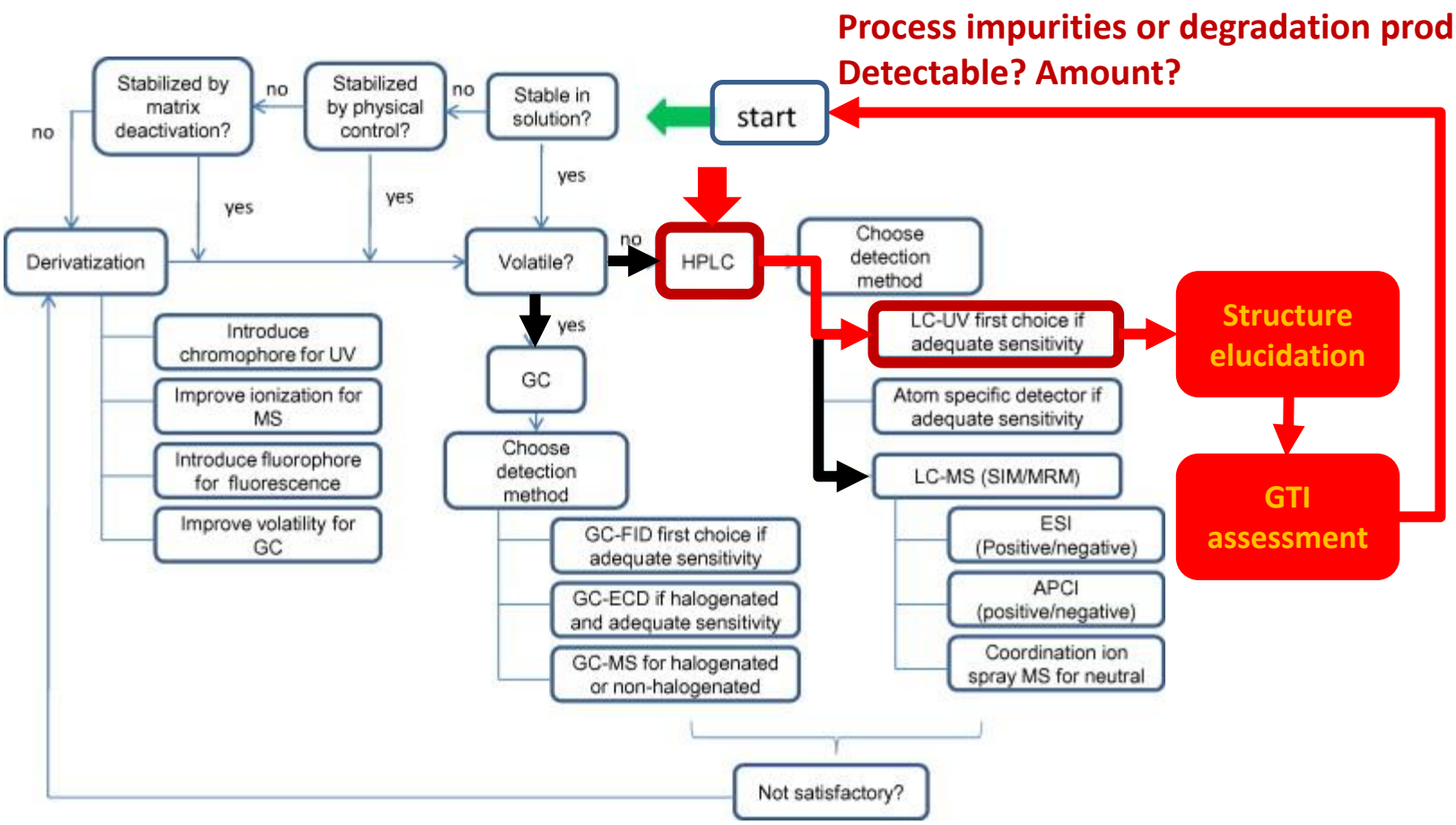



Fig. A decision tree for systematic method development for designing methods for analysis of genotoxic impurities.

TrAC Trends in Analytical Chemistry Volume 49, September 2013, Pages 108-117

# NDMA contamination

- Since the 2018 recall of a single lot of valsartan, there have been recalls or warnings issued various sartan drug lots due to nitrosamine contamination in the drug substance.
- In late 2019, NDMA contamination in ranitidine as a degradant, resulted in removement all ranitidine products from the market.
- In Feb of 2020, NDMA was identified in metformin products, prompting recalls of the products.

# FDA-published testing methods to provide options for regulators and industry to detect NDMA and NDEA impurities

- Combined headspace method: a GC/MS method that allows determination of both N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA) simultaneously
- Combined direct injection method: a GC-MS/MS method that allows for determination of both NDMA and NDEA simultaneously
- Direct injection GC-MS method: a method that can detect NDMA, NDEA, N-Nitrosodiisopropylamine (NDIPA), N-Nitrosoethylisopropylamine (NEIPA), and N-nitrosodibutylamine (NDBA)
- Headspace GC-MS method: a method that can detect NDMA, NDEA, NDIPA, and NEIPA
- LC-HRMS method: a method that can detect NDMA, NDEA, NEIPA, NDIPA, NDBA, and N-Nitroso-N-methyl-4-aminobutyric acid (NMBA)
- RapidFire-MS/MS method: a method that can detect NEIPA, NDIPA, NDBA, and NMBA. We do not recommend using this method to detect NDMA or NDEA because it is less sensitive to those impurities.

https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-angiotensin-ii-receptor-blocker-arb-recalls-valsartan-losartan

# Methods for determination of nitrosamines Provided by OMCLs of the General European Network

- LGL method: **LC-MS/MS** method for the quantitative determination of NMBA in losartan drug substances.
- Swissmedic limit test for the determination of Nitrosamines by **GC-MS/MS** is validated for the following sartan preparations (valsartan, losartan, irbesartan, olmesartan and candesartan).
- CVUA Karlsruhe method is based on **UHPLC-APCI-MS/MS** and allows determination of NDMA and NDEA in sartan drug substances and drug products.
- PALG method is based on **Headspace GC-MS** and applicable to the determination of NDMA in drug substances and corresponding powdered tablets of the sartan group.
- ANSM method is based on **HPLC-UV** and applicable to the determination of NDMA and NDEA in sartan drug.
- This is to method is based on **HPLC-UV** and applicable to the determination of NDMA in drug substance and corresponding powdered tablets of valsartan.

https://www.edqm.eu/en/ad-hoc-projects-omcl-network

# Methods for determination of nitrosamines (OMCL network)

| Analytical technique | GC-MS/MS (DI) | GC-MS/MS (HS) | LC-MS/MS | LC-UV |
|---|---|---|---|---|
| Analyte(s) | NDMA and NDEA | | | |
| Workup procedure | Extraction with MeOH or DCM; LLE with NaOH and DCM | Direct HS-analysis after dissolution in NMP or DMSO | Extraction with MeOH | Extraction with MeOH/, $H_2O$ (35:65 V/V) |
| DS | valsartan<br>irbesartan<br>losartan<br>candesartan<br>olmesartan | valsartan<br>irbesartan<br>losartan<br>candesartan<br>olmesartan | valsartan<br>irbesartan<br>losartan<br>ranitidine | valsartan<br>irbesartan<br>losartan<br>candesartan<br>olmesartan |
| NDMA – LOD | 0.002-0.01 ppm (DS) | 0.005-0.04 ppm (DS) | 0.010-0.15 ppm (DS) | 0.02-0.10 ppm |
| NDMA – LOQ | 0.005-0.05 ppm (DS) | 0.1 ppm (DS) | 0.08-0.5 ppm (DS) | 0.04-0.25 ppm |

# Requirements in analysis of GTIs

- Sample preparation to avoid degradation and dissipation to lost
- Separation of target analytes
- Detection of target analytes
- Structural analysis of target analytes
- Sensitive quantification with IS
- Precision and accuracy

Estimated Dose: 30 mg/person/day, Dose duration: > 12M, TTC: 1.5 μg/day.

=>In drug development, GTIs of > 0.005% should be quantified

# Limit of quantitation (LoQ) for nitorosamines

In routine control, LoQ of the employed analytical method should be at or below the limit for the respective impurity.

Justifying skip testing, the LoQ of the analytical procedure employed should be ≤ 30% of the limit.

Justifying omission of specification, the LoQ of the analytical method employed should be ≤ 10% of the limit.

# Additional requirement in selectivity

Different analytical methods may be used for determination of multiple nitrosamines. If the same analytical method is used for multiple nitrosamines, the selectivity of the method should be demonstrated at the LoQ for each nitrosamine.

Assessment report (Procedure number: EMEA/H/A-5(3)/1490)

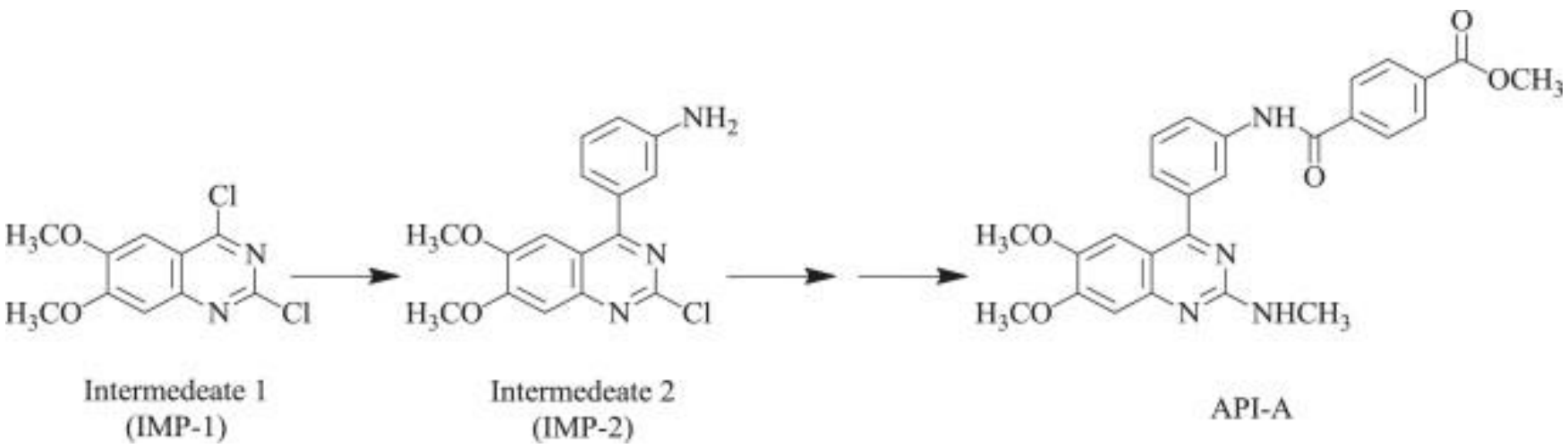
# Outline

1. Introduction
2. Analytical methods for genotoxic impurity (GTI)
3. Examples of analyzing GTIs in drug development

   Case 1: Potential GT Intermediates and co-eluted trace impurities in project A

   Case 2: Lot-to-lot inconsistency in Ames assay caused by trace by-product in project B
4. Challenges in analysis of GTIs
5. Conclusion

# Case 1: PGTIs, synthetic intermediates




pKa of conjugate acid: 5.0
log Po/w: 4.2 (Clog P: 5.3)

Fig. Synthesis of API-A.



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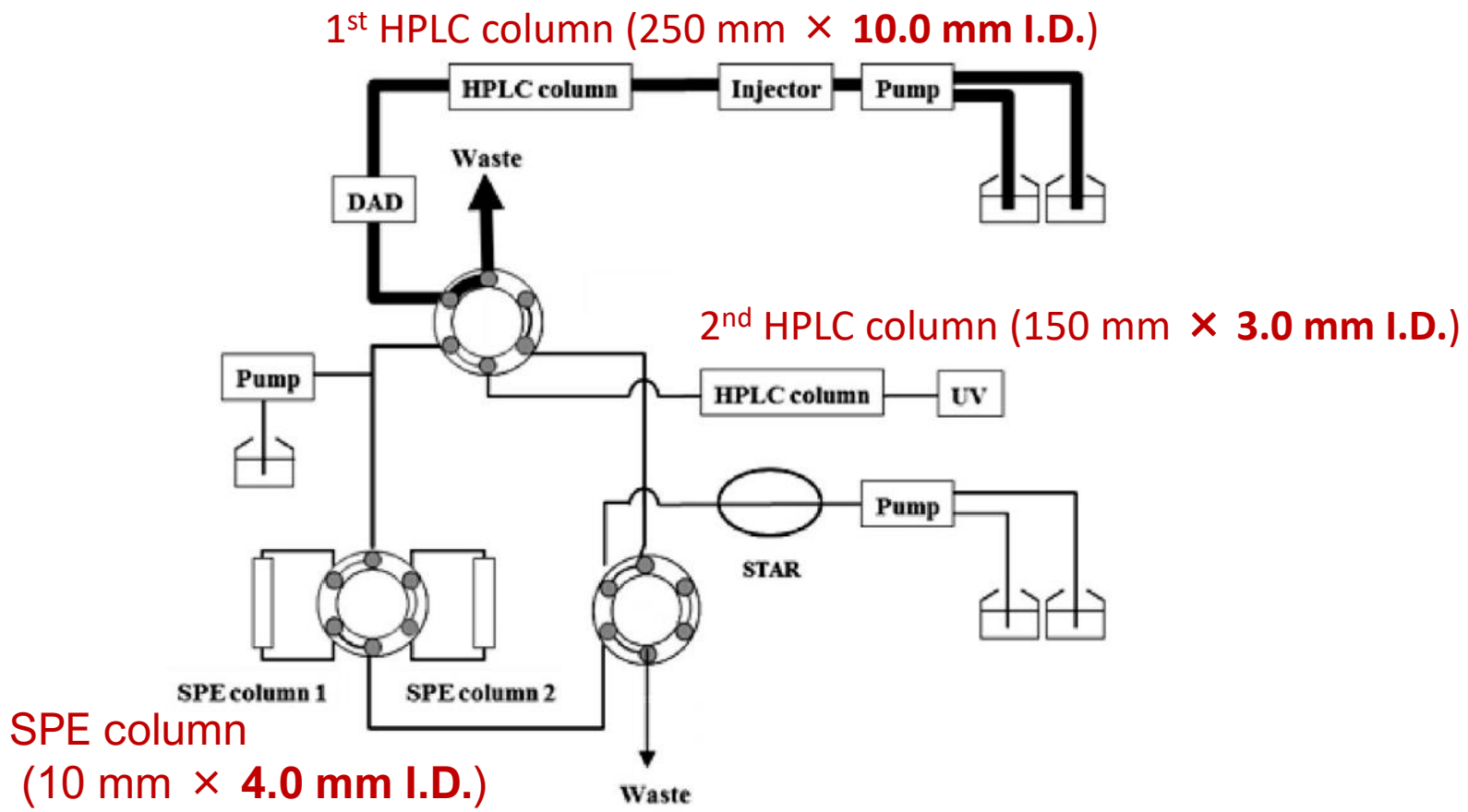
# Two-dimensional HPLC (HPLC-SPE-HPLC) system for sensitive determination of impurities




The two-dimensional HPLC system achieves a stepwise downsizing in HPLC.
Trace components in the sample were concentrated, separated and subsequently detected with high sensitivity.

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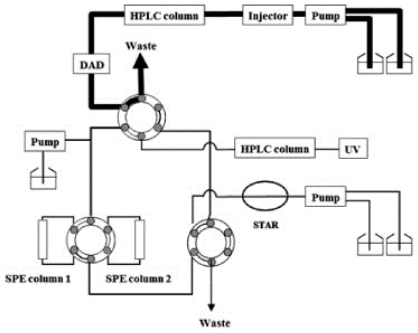
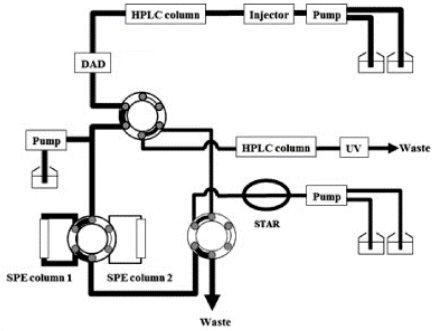
# 1st HPLC and extraction of analytes

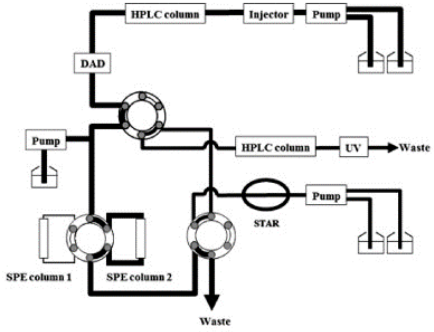
1. Separation on 1st HPLC column




2. Separation on 1st HPLC column, Ext of IMP-2 on SPE column 1




3. Separation on 1st HPLC column, Ext. of IMP-1 on SPE column 2



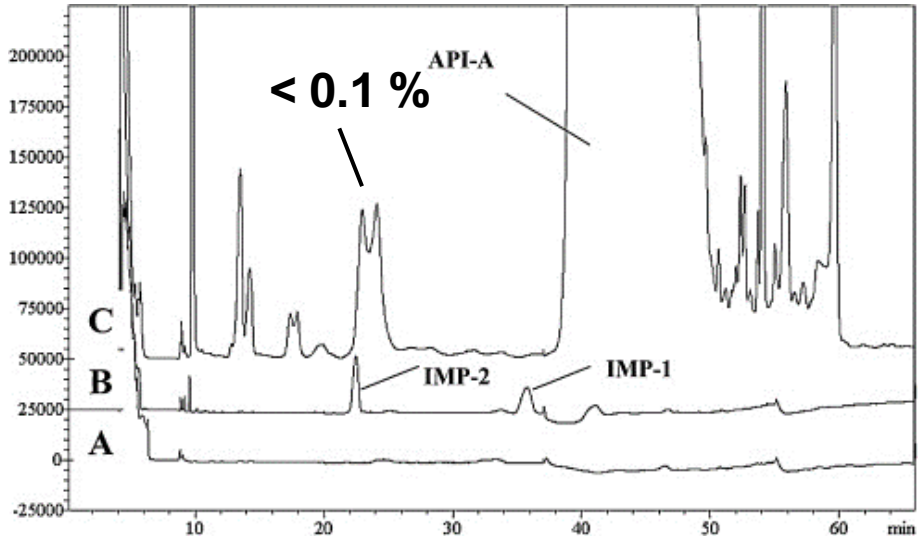




**Fig. Representative HPLC chromatogram of (A) blank (DMSO), (B) IMP-1 and IMP-2 standard solution (3 µg/mL) and (C) API-A (40 mg/mL) in the 1st HPLC. API-A was dissolved in DMSO at 40 mg/mL.**

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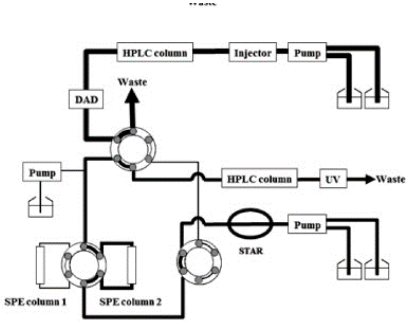
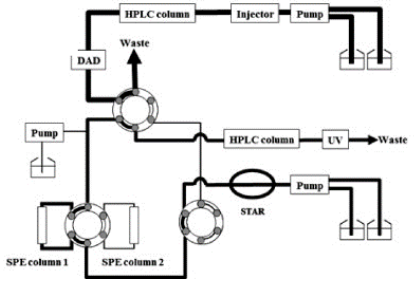
# 2nd HPLC for analysis

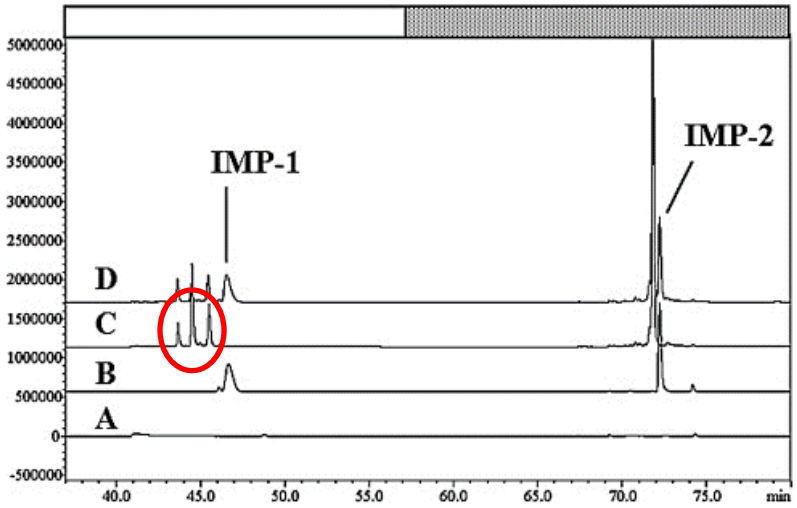
Conditioning of 1st HPLC, analysis of components on SPE column 2 in 2nd HPLC




Conditioning of 1st HPLC, analysis of components on SPE column 1 in 2nd HPLC




Analysis for SPE column 2 Analysis for SPE column 1



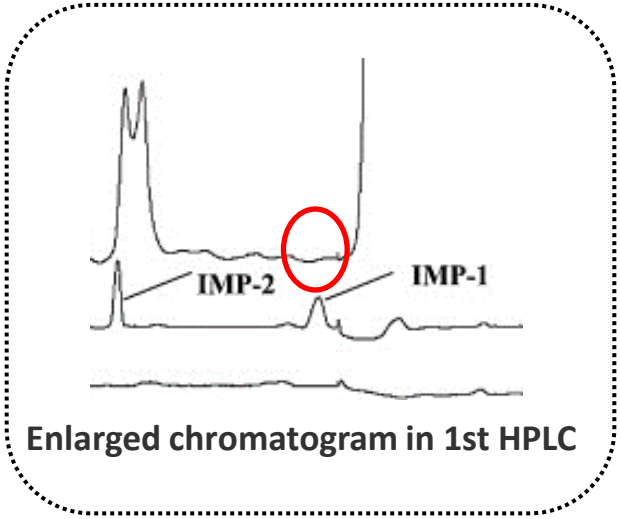




Fig. Representative HPLC chromatogram of (A) blank, (B) standard solution (3 µg/mL), (C) API-A and (D) API-A spiked with IMP-1 and IMP-2 (3 µg/mL, 75 ppm) in the 2nd HPLC.

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# Batch analysis

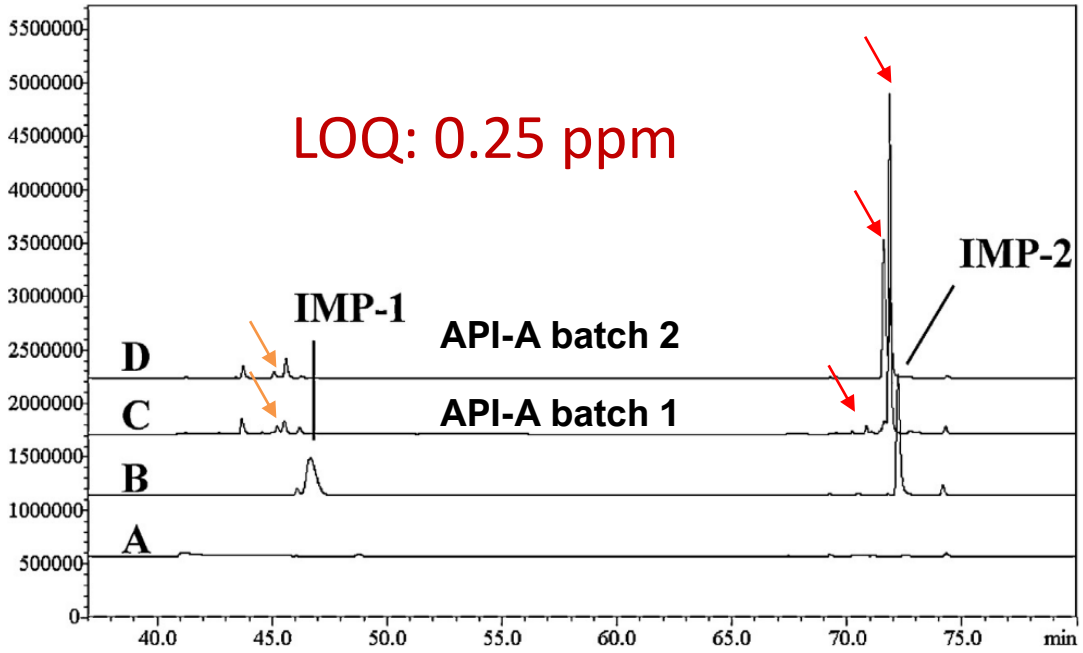
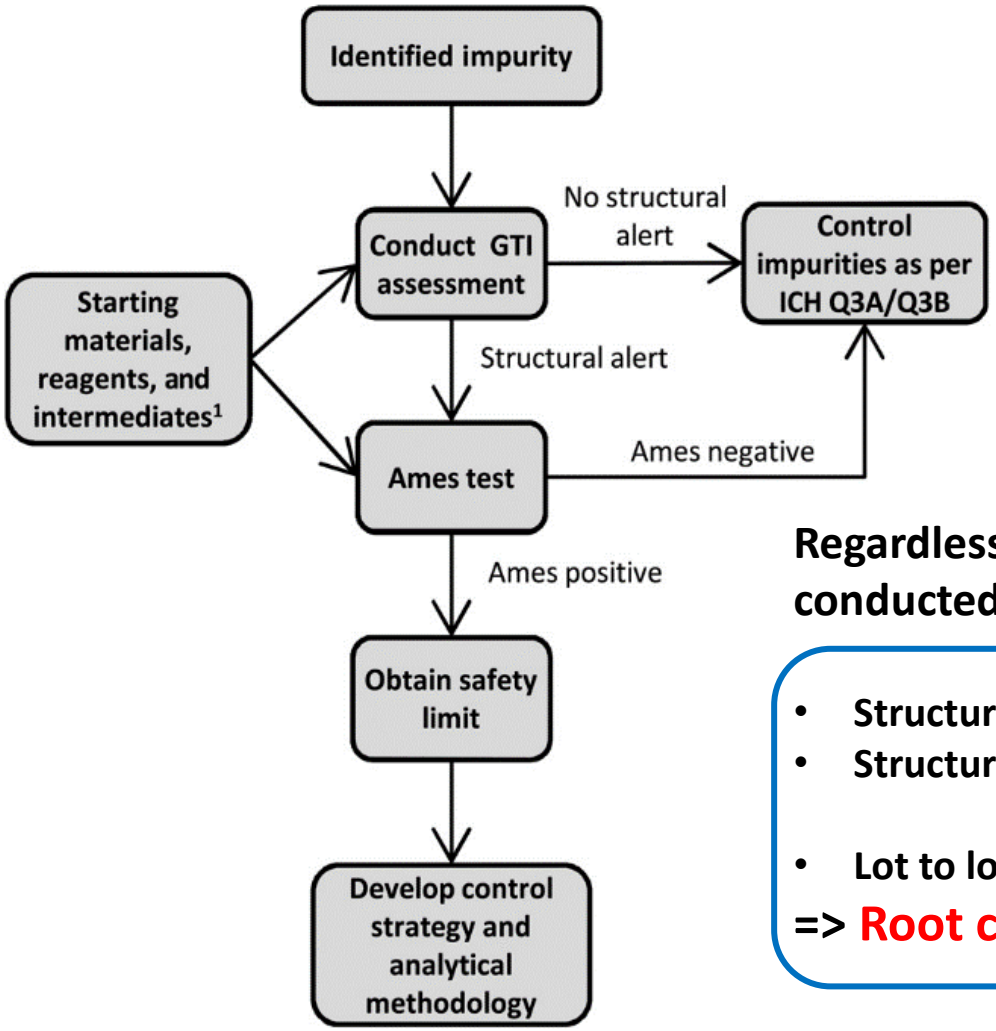



Fig. Representative HPLC chromatogram of (A) blank (DMSO), (B) IMP-1 and IMP-2 standard solution (3 μg/mL, 75 ppm) and (C) API-A batch 1 and (D) API-A batch 2 in the 2nd HPLC.

Both IMP-1 and IMP-2 were NOT detected in these batches, while some trace impurities were found in the chromatogram. It was indicated that IMP-1 and IMP-2 are reactive intermediates, and they did not remain in API-A as they were.

# Case 2: Equivocal, lot to lot inconsistency in Ames test in Project B




**Regardless of in-silico results, Ames testing will be conducted for workspace safety.**

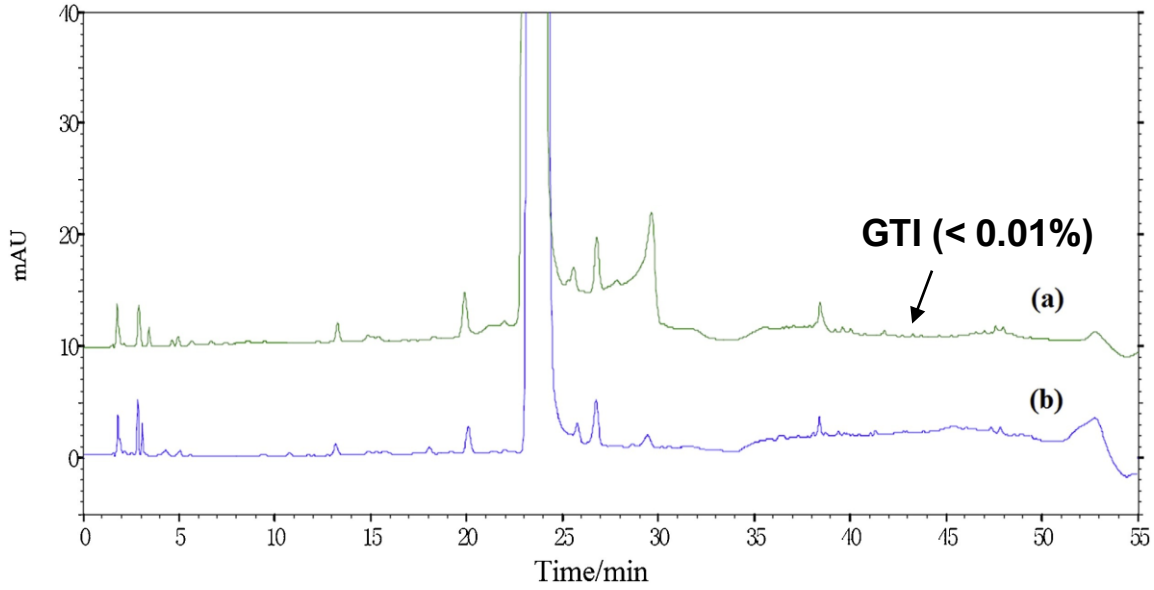
- **Structural alert for API => Negative**
- **Structural alert for identified impurity by DEREK => Negative**
- **Lot to lot consistency in Ames test => Failed**

**=> Root cause analysis**

Figure 3. GTI assessment process for drug substance and drug product.

**J. Pharm. Sci., VOL. 102, NO. 5, MAY 2013**

# Case 2: Equivocal, lot to lot inconsistency in Ames assay




**Virtual LC-UV chromatogram**

## Root cause analysis and related investigations

1. Synthesis of multiple batches to alter impurity profiles
2. Investigation on impurity profile by LC-UV
3. Ames assay for batches
4. Correlation analysis
5. Isolation of candidate impurities
6. Structure elucidation for candidate impurities (even if < 0.01%)
7. Ames assay for candidate impurities
8. Investigation on control strategy
9. Avoiding the contamination of the GTI

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1. Introduction
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# Analytical Challenges

- Potential low limits
  - Dose number
  - Dose duration
- Matrix interference
  - Low concentration of target analytes
  - High conc API
- Diverse physico-chemical properties
  - Highly reactive
  - Unstable
  - Non-chromophoric in LC and/or non-volatile in GC

# Analytical challenges

- Development of generic method e.g., nitrosamines, sulfonate esters
- Improvement in accuracy and precision
- Analysis of drug-related GTIs in marketed products including generic drugs

# A Cautionary Tale: Quantitative LC-HRMS Analysis of NDMA in Metformin

**Table 2.** Comparison of mass spectrometry (MS) conditions used in this study (FDA) and the private laboratory method description

| MS Conditions | Private laboratory | FDA |
|---|---|---|
| Instrument | QToF | Orbitrap |
| Ionization mode | APCI, positive | APCI, positive |
| Data acquisition | MRMHR | Targeted MS2 |
| MS scan | 50–450 m/z | 40–90 m/z |
| Mass resolution | >25,000[a] | 45,000[b] |
| Transition(s) | 75.0553 → 75.0553 | 75.0553 → 75.0553 |
| | 83.0997 → 83.0997 | 83.0997 → 83.0997 |

[ab] The maximum resolution is specified as ≥ 42,000 (FWHM) at *m/z* 956 for this instrument; The maximum resolution is specified as 480,000 at *m/z* 200 for this instrument

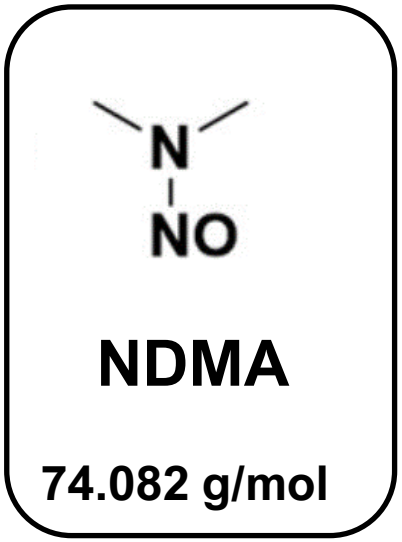
**Table 1.** NDMA Amounts in metformin samples reported by FDA (using FDA-1 and FDA-2 methods) and the private laboratory

| Sample # | Metformin dosage and formulation | Manufacturer name as per private laboratory | Lot # | FDA-1[a,b] (ng/mg) | FDA-2 (ng/mg) | Private lab (ng/mg) |
|---|---|---|---|---|---|---|
| 1 | 500 mg IR | ACI Healthcare USA, Inc. | D105061 | ND[c] | ND | 0.062 |
| 2 | 500 mg IR | ACI Healthcare USA, Inc. | C105019A | ND | ND | ND |
| 3 | 500 mg IR | ACI Healthcare USA, Inc. | D105019 | ND | ND | ND |
| 4 | 500 mg ER | Actavis Pharma, Inc. | 1376339 M | 0.021[d] | 0.021 | 0.364 |
| 5 | 750 mg ER | Actavis Pharma, Inc. | 1354471A | 0.050 | 0.047 | 0.427 |
| 6 | 500 mg ER | Aiping Pharmaceutical, Inc. | 190300211 | ND | ND | ND |
| 7 | 1000 mg ER | Aiping Pharmaceutical, Inc. | 190200411 | ND | 0.008 [d] | ND |
| 8 | 1000 mg IR | Zydus | 184759 | ND | 0.006 [d] | ND |
| 9 | 750 mg ER | Amneal Pharmaceuticals, LLC | AM180770A | 0.079 | 0.076 | 0.600 |
| 10 | 500 mg ER | Amneal Pharmaceuticals, LLC | AM190107AA | 0.314 | 0.292 | 0.790 |
| 11 | 500 mg ER | Amneal Pharmaceuticals, LLC | HD03319A | 0.293 | 0.255 | 0.566 |
| 12 | 500 mg ER | Amneal Pharmaceuticals, LLC | HM02918A | 0.289 | 0.265 | 0.564 |
| 13 | 850 mg IR | Amneal Pharmaceuticals, LLC | AM180405A | ND | ND | 0.276 |
| 14 | 500 mg ER | Apotex Corp. | NE5801 | 0.121 | 0.112 | 0.180 |
| 22 | 500 mg ER | Granules Pharma Inc | 4910134A | ND | ND | 0.082 |
| 23 | 850 mg IR | Heritage | 4510157A | ND | ND | 0.299 |
| 24 | 500 mg IR | Heritage | 4500753A | ND | ND | 0.412 |
| 25 | 1000 mg IR | Heritage | 4521630A | ND | ND | ND |
| 26 | 500 mg ER | Ingenus Pharmaceuticals | 19388005 | 0.012 [d] | 0.009 [d] | ND |
| 27 | 500 mg ER | Lupin Pharma | G901203 | 0.170 | 0.138 | 0.244 |
| 28 | 1000 mg IR | Megalith Pharmaceuticals | 442180318 | ND | ND | ND |
| 29 | 1000 mg ER | Mylan Pharmaceuticals | 3090719 | 0.011 [d] | 0.010 | ND |
| 30 | 1000 mg ER | Nostrum Labs Inc | MEF290206 | ND | ND | ND |
| 31 | 500 mg ER | Oceanside | 19D125P | 0.010 [d] | 0.005 [d] | ND |
| 32 | 750 mg ER | Sun Pharmaceutical Ind | JKU0880A | ND | ND | ND |
| 33 | 500 mg ER | Sun Pharmaceutical Ind | JKU2539A | ND | ND | ND |
| 34 | 500 mg ER | Tagi Pharma Inc | 5841,910035 | ND | ND | ND |
| 35 | 500 mg ER | Tagi Pharma Inc | 5841905129 | 0.015 [d] | 0.012 | ND |
| 36 | 500 mg ER | Time Cap Laboratories | XP9004 | 0.082 | 0.071 | 0.106 |
| 37 | 500 mg IR | Westminster Pharmaceuticals | B105067B | ND | ND | ND |
| 38 | 1000 mg IR | Westminster Pharmaceuticals | B107261B | ND | ND | ND |


| Sample # | Metformin dosage and formulation | Manufacturer name as per private laboratory | Lot # | FDA-1[a,b] (ng/mg) | FDA-2 (ng/mg) | Private lab (ng/mg) |
|---|---|---|---|---|---|---|
| 13 | 850 mg IR | Amneal Pharmaceuticals, LLC | AM180405A | ND | ND | 0.276 |
| 14 | 500 mg ER | Apotex Corp. | NE5801 | 0.121 | 0.112 | 0.180 |

# Co-elution of NDMA and DMF in LC

## N,N-Dimethylformamide (DMF): Residual Solvent Class 2




**NDMA**

**74.082 g/mol**


**DMF**

**73.094 g/mol**

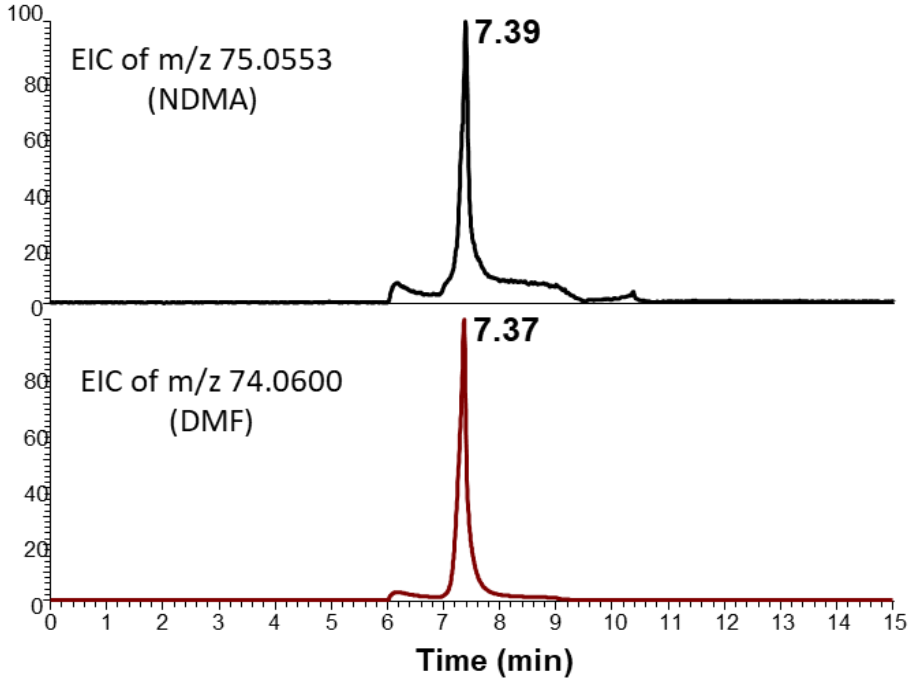



Fig. Co-elution of NDMA and DMF in the chromatography used by the private laboratory. The EIC of the exact mass of NDMA (eluting at 7.39 min) and EIC of the exact mass of DMF (eluting at 7.37 min) are indicated in the Figure.

# Possible MS interference of DMF to NDMA in Metformin

DMF monoisotopic ion at m/z 74.0597

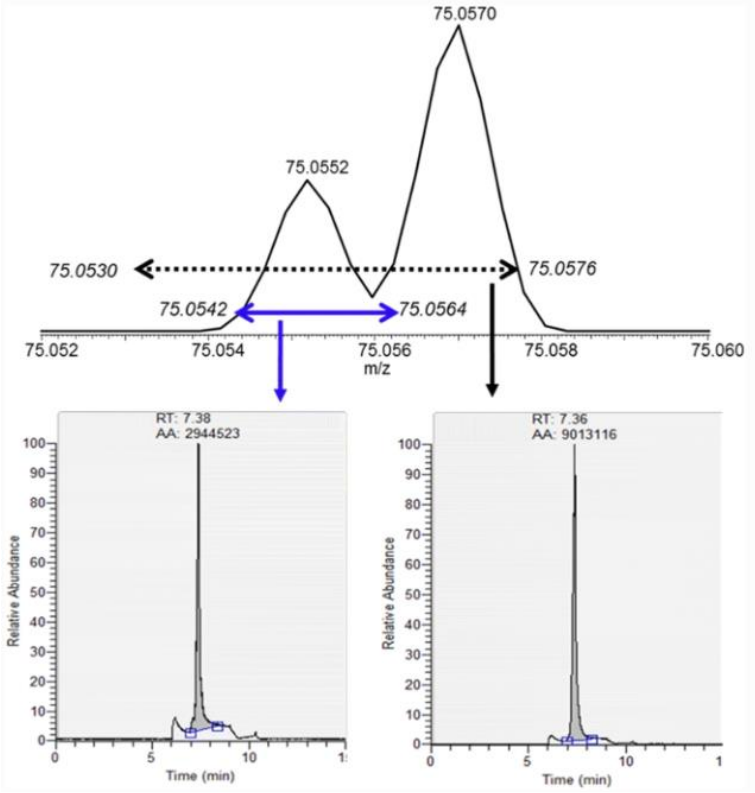



Fig. Mass spectra of Sample #13 (ER drug product) spiked with 20 ng/mL of NDMA which also contained DMF (top) and EICs (bottom) demonstrating the overestimation (integrated area of 2,944,523 with ± 15 ppm mass tolerance in the left panel (blue bar), while there is an integrated area of 9,013,116 with ± 30 ppm mass tolerance in the right panel (dotted bar)) of NDMA as the results of DMF interference from $C_3H_7NO$

DMF $^{15}N$ isotopic ion can be mistakenly identified as the NDMA ion

The AAPS Journal (2020) 22:89

# Formation of NDMA from ranitidine samples by heat in headspace-GC system

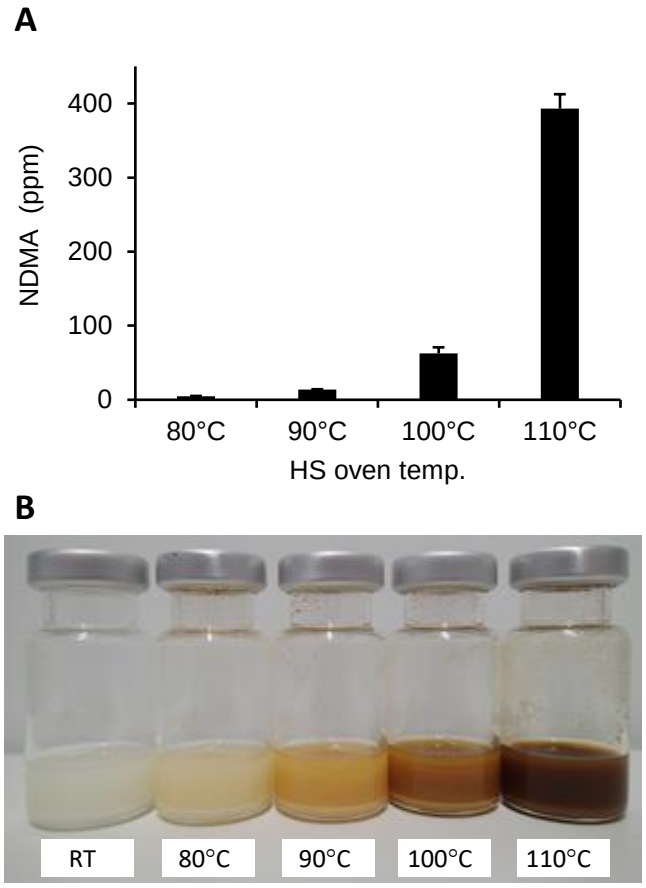



**Fig. NDMA formation in ranitidine tablet sample by 10-min heating at various temperature in Headspace-GC analysis**

(A) Amount of NDMA under various heating condition in the headspace oven. Each result represents the mean ± SD (n=3). (B) Visual appearance changes after 10 min equilibration at various temperatures.

# Conclusion

- Analytical methods for nitrosamines in some drugs have been validated and available for the quality control of marketed drugs
- Analytical methods for other GTIs have not been well established
- Fit-for-purpose and general methods have to be developed for marketed drugs

# Appendices

# Eiichi YAMAMOTO

- Since Oct. 2018, Section chief of drug division in National Institute of Health Sciences (NIHS).
- Over 22 years R&D experience in Eisai Co. Ltd., a pharma.
  - PhysChem & Preformulation
  - Drug substance analysis
  - Drug product analysis
  - Nano drug delivery system research
  - Contributed to more than 10 projects
  - Contributed to 2 NDAs for Pariet® &Halaven®
- Wide range of knowledge in drug discovery and drug development

# My experiences analyzing GTIs

In Eisai,

- Analysis of potential genotoxic synthetic intermediates and by-products in drug substances (DS)
- Analysis of genotoxic degradation products in DS and their drug products (DP)
- Predicting amount of the degradation products in DP

In NIHS,

- Analysis of NDMA in ranitidine, metformin, etc.