PO-CON1548E

SHIMADZU
Excellence in Science

Determination of Leachables in Orally Inhaled and Nasal Drug Products (OINDP) by GCMS/MS

ASMS 2015 TP 311

Prashant Hase, Ankush Bhone, Durvesh Sawant, Dheeraj Handique, Sanket Chiplunkar, Ajit Datar, Jitendra Kelkar and Pratap Rasam
Shimadzu Analytical (India) Pvt. Ltd.,
1 A/B Rushabh Chambers, Makwana Road, Marol, Andheri (E), Mumbai-400059, Maharashtra, India.

Determination of Leachables in Orally Inhaled and Nasal Drug Products (OINDP) by GCMS/MS

Introduction

Orally Inhaled and Nasal Drug Products (OINDP) are developed for treating asthma or chronic obstructive pulmonary diseases by delivering drug substances directly to the respiratory tract. Some of the inhaled drug products are by far the most pharmacologically effective substances that are administered to humans[1]. OINDP are present in inhaler devices. These devices may contain polymers, elastomers and other components from which minute quantities of material may migrate/leach into product and enter the respiratory tract along with the therapeutic agent.

Figure 1. Metered Dose Inhalers

Leaching can be promoted by the formulation, or components of formulation like Chlorofluorocarbon (CFC) or Hydrofluoro Alkanes (HFA) propellants in Metered Dose Inhalers (MDI's) (Figure 1). Due to the time dependent nature of leaching process, leachables in OINDP formulation over the shelf life of the product are determined by appropriate stability studies[2].

Among the leachables, nitrosamines (Figure 2), phthalates (Figure 3) and Poly Aromatic Hydrocarbons (PAH) (Figure 4) were targeted due to their toxicity and carcinogenicity at low concentration levels.

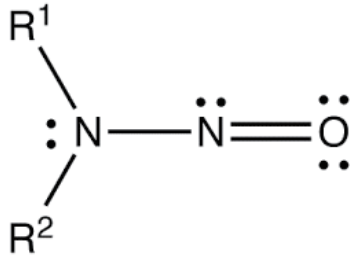


Figure 2. General structure for Nitrosamine

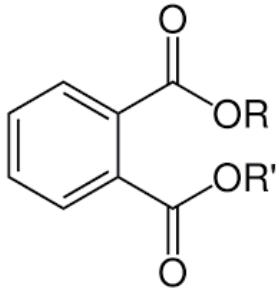


Figure 3. General structure for Phthalates

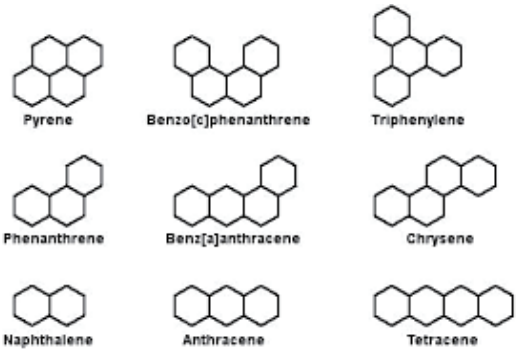


Figure 4. Some of the structures for PAH's

Method of analysis

Extraction of leachables from MDI product

Simple and straight forward Solid Phase Extraction (SPE) technique was employed for sample preparation. MDI with 200 metered doses (Salbutamol Inhalation IP 100 mcg/dose) were procured from local medical store.

Standard mixtures of phthalates (15 compounds), nitrosamine (7 compounds) and PAH (18 compounds) were procured from Restek®.

1. Standard preparation:

Standards stocks of Phthalates [1000 µg/mL in hexane : acetone (80:20 v/v)], nitrosamines (1000 µg/mL in dichloromethane) and PAH's [500 µg/mL in acetonitrile : toluene (92:8 v/v)] were procured. These individual stocks were further mixed together to prepare linearity solutions ranging from 1 ppb to 100 ppb in dichloromethane.

2. Sample preparation:

Sample Matrix	: The inhaler content of 200 actuations was completely transferred in to glass container and added 10 mL LCMS grade water (JT Baker).
SPE Tube	: Orochem® C18, 1000 mg, 6 mL.
Cartridge Conditioning	: 5 mL DCM, 5 mL methanol and 5 mL LCMS grade water (twice each).
Sample Condition	: 10 mL of sample matrix passed through conditioned cartridge
Elution	: 10 mL of DCM
Post treatment	: To 10 mL eluent add 0.5 g of sodium sulfate to remove residual water

The DCM layer was carefully removed and injected into the GCMS-TQ8040 system (Figure 5). Such simple extraction method can be easily employed by pharmaceutical industry.

MRM method development

Individual mixtures of phthalates, nitrosamines and PAH standards were procured from Restek®. For MRM optimization, about 5 ppm individual standard mixtures were analyzed separately using scan mode. Standard mixture of n-alkane were run to set the retention index of targeted compounds. For individual components, precursor ions were selected. Using selected precursor ions, product ion scan was performed with different Collision Energies (CE) (Refer Figure 6). For each component, MRM transitions with appropriate CEs were determined. For PAH already available MRM transitions and optimized CEs were used. All the above steps were simplified with the help of Smart MRM optimization tool. These MRM transitions along with individual retention index were registered to Smart Database and the final MRM method with optimum segments (Refer Figure 7) was generated (Refer Table 2).

Figure 5. GCMS-TQ8040 Triple quadrupole system by Shimadzu

Determination of Leachables in Orally Inhaled and Nasal Drug Products (OINDP) by GCMS/MS

Key Features of GCMS-TQ8040

- ASSP™ (Advanced Scanning Speed Protocol) enables high-speed scan and data acquisition for accurate quantitation at 20,000 u/sec.
- Capable of performing simultaneous Scan/MRM.
- UFsweeper® technology efficiently sweeps residual ions from the collision cell for fast, efficient ion transport ensuring no cross-talk.
- Two overdrive lenses reduce random noise from helium, high-speed electrons and other factors to improve S/N ratio.
- Flexible platform with EI (Electron Ionization), PCI (Positive Chemical Ionization), and NCI (Negative Chemical Ionization) techniques.
- Full complement of acquisition modes including MRM, Scan/MRM, Precursor Ion, Product Ion and Neutral Loss Scan.

GCMS/MS Analytical Conditions

The analysis was carried out on Shimadzu GCMS-TQ8040 as per the conditions given in Table 1.

Table 1. Analytical conditions

Chromatographic parameters

Column	: Rxi-5Sil MS (30 m L x 0.25 mm I.D. x 0.25 µm)
Injection Mode	: Splitless
Sampling Time	: 2.0 min
Split Ratio	: 10.0
Carrier Gas	: Helium
Flow Control Mode	: Linear Velocity
Linear Velocity	: 36.1 cm/sec
Column Flow	: 1.0 mL/min
Injection Volume	: 3.0 µL
Injector Type	: Programmed Temperature Vaporization (PTV)
Total Program Time	: 55.00 min

Column Temp. Program :

Rate (°C /min)	Temperature (°C)	Hold time (min)
	40.0	5.00
10.00	200.0	2.00
5.00	300.0	12.00

Injector Temp. Program :

Rate (°C /min)	Final Temperature (°C)	Hold time (min)
	40.0	0.50
250.00	280.0	53.50

Mass Spectrometry parameters

Ion Source Temp.	: 230.0 °C
Interface Temp.	: 280.0 °C
Ionization Mode	: EI
Acquisition Mode	: MRM

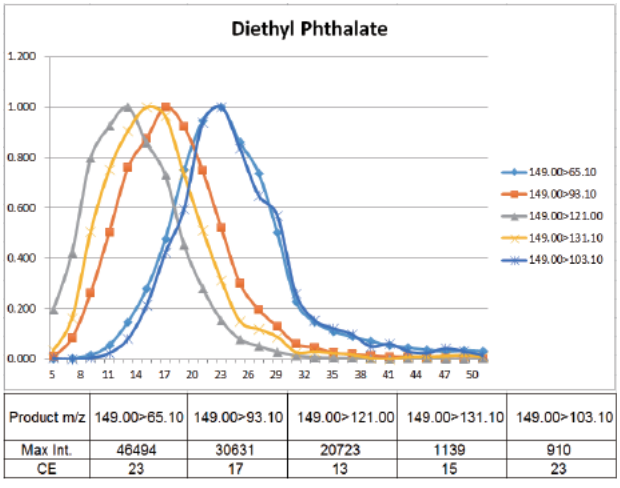
Determination of Leachables in Orally Inhaled and Nasal Drug Products (OINDP) by GCMS/MS

Results

The mixture of nitrosamines, PAH and phthalates was analyzed using the method created as mentioned in Table 2. The leachables were quantitatively extracted from sample were evaluated statistically with respect to linearity and precision.

Table 2. Method creation using GCMS-TQ8040 Smart MRM

Type of compounds	Condition	Intermediate requirement	Step.1	Step.2	Step.3
Phthalates	No information about transitions	Measure in SCAN mode and determine Pre-cursor ion	MRM Optimization Tool Create Batch sequence and Method file of several Collision Energy automatically.	MRM Optimization Tool Analyzes acquired data files and selects the best transitions and collision energy automatically. The result can be exported and by single click Smart MRM database for leachable was created.	Method creation using Smart MRM Database
PAH	Known MRM transitions but, Collision energies are not optimized	→			
Nitrosamines	Known MRM transitions but, Collision energies are not optimized	→			



Product m/z	149.00>65.10	149.00>93.10	149.00>121.00	149.00>131.10	149.00>103.10
Max Int.	46494	30631	20723	1139	910
CE	23	17	13	15	23

Figure 6. CE Optimization using Smart MRM optimization tool

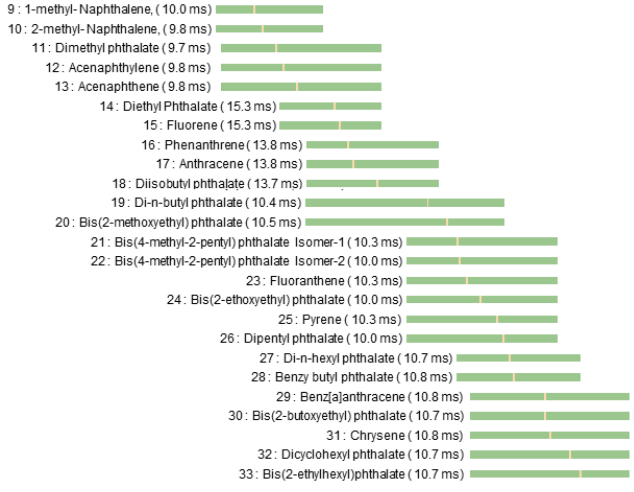


Figure 7. Optimum segmented method created using Smart Database

Relative Standard Deviation (% RSD) for 5 ppb Nitrosamines standard solution (n=6) and 10 ppb Phthalates and PAH's was less than 10% for all components (Refer Table 3). Linearity plot for standards ranging from 1 ppb to 100 ppb concentration level showed linear response with r2 0.995 to 0.999 (Refer Figure 8-10). The content of Phthalates, Nitrosamines and PAH in 5 samples (Salbutamol Inhalation IP 100 mcg/dose 200 metered dose) was found to be below detection limit. On the basis of statistical data obtained, the method was proved to be highly selective, sensitive, linear and reproducible.

Determination of Leachables in Orally Inhaled and Nasal Drug Products (OINDP) by GCMS/MS

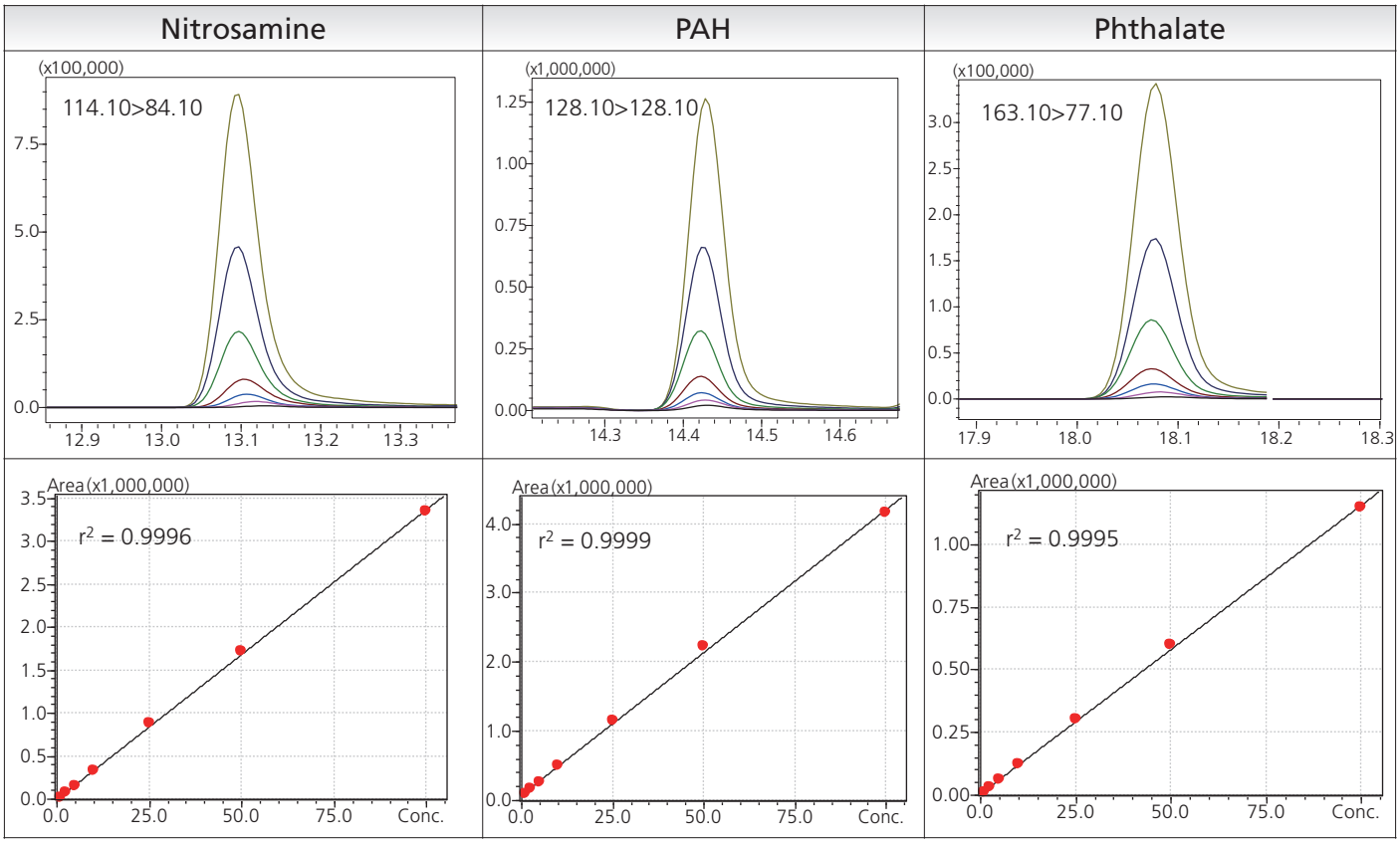


Figure 8. Overlay and linearity for N-Nitrosopiperidine

Figure 9. Overlay and linearity for Naphthalene

Figure 10. Overlay and linearity for Dimethyl Phthalate

Determination of Leachables in Orally Inhaled and Nasal Drug Products (OINDP) by GCMS/MS

Table 3. Quantitation results

ID	Group	Name	RT (min)	Index	Calibration Range (ppb)	r^2	*LOQ (ppb)	*LOD (ppb)	% RSD (n=6)
1	Nitrosamine	N-Nitrosodimethylamine	5.24	712	2.5.-100	0.9923	0.9	0.3	6.2
2		N-Nitrosomethyethylamine	7.32	844	2.5 - 100	0.9996	0.4	0.1	4.6
3		N-Nitrosodiethylamine	8.94	904	1 - 100	0.9999	4.4	1.5	5.4
4		N-Nitrosopyrrolidine	12.31	1067	2.5 - 100	0.9999	2.8	0.9	4.0
5		N-Nitrosodi-n-propylamine	12.41	1072	1 - 100	0.9998	3.4	1.1	7.9
6		N-Nitrosopieridine	13.09	1110	1 - 100	0.9995	1.7	0.6	5.1
7	PAH	Naphthalene	14.43	1191	1 - 100	0.9985	1.7	0.5	2.2
8	Nitrosamine	N-Nitrosodi-n-butylamine	15.49	1262	1 - 100	0.9997	4.2	1.4	5.6
9	PAH	1-methyl- Naphthalene,	16.11	1304	1 - 100	0.9990	1.5	0.5	2.0
10		2-methyl- Naphthalene,	16.34	1320	1 - 100	0.9986	1.5	0.5	2.0
11		Dimethyl phthalate	18.08	1449	1 - 100	0.9996	2.1	0.7	2.7
12		Acenaphthylene	18.26	1463	1 - 100	0.9993	1.7	0.6	2.2
13		Acenaphthene	18.69	1496	1 - 100	0.9998	1.6	0.5	2.0
14		Diethyl Phthalate	19.81	1587	1 - 100	0.9975	1.6	0.5	2.1
15		Fluorene	19.95	1598	1 - 100	0.9995	1.6	0.5	2.4
16		Phenanthrene	22.43	1801	1 - 100	0.9998	1.9	0.6	2.6
17		Anthracene	22.59	1811	1 - 100	0.9991	2.2	0.7	3.0
18	Phthalates	Diisobutyl phthalate	23.28	1856	1 - 100	0.9986	1.9	0.6	2.6
19		Di-n-butyl phthalate	24.81	1951	1 - 100	0.9995	1.7	0.5	2.2
20		Bis(2-methoxyethyl) phthalate	25.38	1985	1 - 100	0.9999	5.2	1.7	6.8
21		Bis(4-methyl-2-pentyl) phthalate Iso-1	26.62	2058	1 - 100	0.9999	4.1	1.3	5.0
22		Bis(4-methyl-2-pentyl) phthalate Iso-2	26.68	2062	1 - 100	0.9995	3.2	1.1	4.2
23	PAH	Fluoranthene	26.89	2074	1 - 100	0.9999	1.4	0.5	1.8
24	Phthalates	Bis(2-ethoxyethyl) phthalate	27.30	2098	5 - 100	0.9999	5.3	1.7	5.9
25	PAH	Pyrene	27.79	2128	1 - 100	0.9999	1.1	0.4	1.4
26	Phthalates	Dipentyl phthalate	27.99	2139	1 - 100	0.9999	1.9	0.6	2.9
27		Di-n-hexyl phthalate	31.18	2330	1 - 100	0.9998	2.9	1.0	3.5
28		Benzy butyl phthalate	31.29	2337	1 - 100	0.9986	4.0	1.3	5.2
29	PAH	Benz[a]anthracene	33.22	2456	1 - 100	0.9975	5.2	1.7	7.2
30	Phthalates	Bis(2-butoxyethyl) phthalate	33.21	2456	2.5 - 100	0.9997	4.3	1.4	5.6
31	PAH	Chrysene	33.37	2466	1 - 100	0.9983	3.9	1.3	5.7
32	Phthalates	Dicyclohexyl phthalate	33.97	2504	1 - 100	0.9994	2.3	0.8	2.9
33		Bis(2-ethylhexyl)phthalate	34.27	2523	2.5 - 100	0.9991	3.3	1.1	4.0
34		Di-n-octyl phthalate	37.18	2719	1 - 100	0.9989	4.0	1.3	6.3
35	PAH	Benzo[b]fluoranthene	37.89	2768	1 - 100	0.9943	3.8	1.2	6.0
36		Benzo[k]fluoranthene	38.09	2776	1 - 100	0.9969	4.6	1.5	5.7
37		Benzo[a]pyrene	39.15	2858	1 - 100	0.9993	10.0	3.0	5.9
38		Dinonyl phthalate	39.93	2915	1 - 100	0.9988	4.6	1.5	5.8
39		Indeno[1,2,3-cd]pyrene	43.27	3170	1 - 100	0.9946	10.0	3.0	7.9
40		Dibenz(a,h)anthracene	43.43	3183	1 - 100	0.9940	7.3	2.4	8.7
41		Benzo[g,h,i]Perylene	44.17	3236	2.5 - 100	0.9985	8.5	2.8	6.9

*As per software calculation

Determination of Leachables in Orally Inhaled and Nasal Drug Products (OINDP) by GCMS/MS

Conclusions

- A highly sensitive method was developed for quantitation of 40 leachables in OINDP products by using Shimadzu GCMS-TQ8040.
- The MRM method developed for about 40 leachables can be used for screening of leachables in various Pharmaceutical products.
- Ultra Fast scanning, UFsweeper® and ASSP™ features enabled sensitive, selective, fast, reproducible, and linear method of analysis.

References

[1] Ball D, Blanchard J et al., Toxicological Sciences Volume 97 (2), (2007), 226-236.
[2] Safety threshold and best practices for extractables and leachables in orally inhaled and nasal drug products (2006), Product Quality Research Institute (PQRI).

First Edition: May, 2015

SHIMADZU
Shimadzu Corporation
www.shimadzu.com/an/