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Application News

Gas Chromatograph-Flame Ionization Detector Nexis™ GC-2030

Fast - GC Method for Residual Solvent Analysis in Drug Discovery Workflow

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User Benefits

- The method provides high throughput required by drug discovery industry for analyzing multiple solvents in short run time of 8 minutes.
- Active time management feature enables user to automate functions like start, shutdown & conditioning of system.

■ Introduction

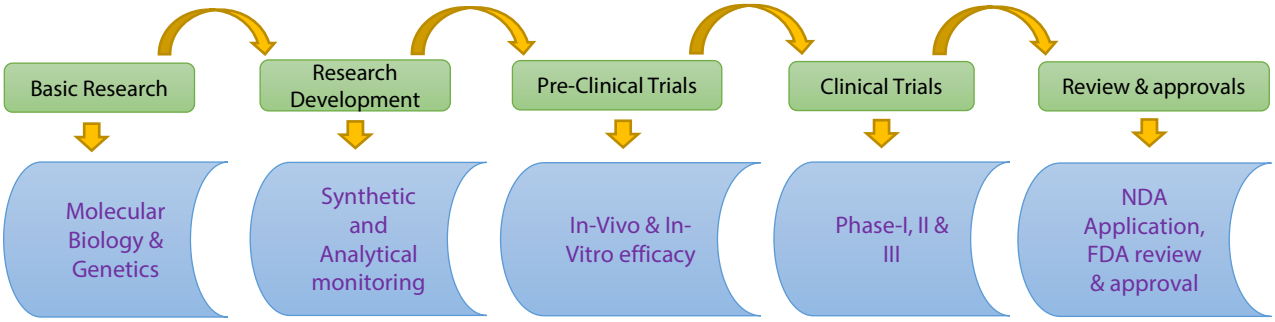

Fig. 1 Drug Discovery Workflow

Overview : Drug discovery is the process through which potential new medicines are identified. This process includes target identification to identify potential drug molecule, such as proteins or genes involved in disease, and validate their therapeutic potential. Refer Fig. 1 for stepwise workflow. To address the growing burden of chronic diseases, there is a need for the development of new and effective treatments. As a result, there is an increasing demand for drug discovery laboratories to reduce the expenses and duration for development of new drugs. Hence, those techniques play an important role which deliver high throughput with reduced operation cost.

Residual Solvent : Residual solvents in pharmaceuticals are defined as organic volatile chemicals that are used or produced in the manufacturing of drug substances or excipients, or in the preparation of drug products. The solvents are not completely removed by practical manufacturing techniques.
Residual solvent analysis is used to ensure pharmaceuticals are free from toxicologically significant levels of volatile organic compounds either left behind or created in the manufacturing process. Also, these solvents could influence the efficacy and degradation of drugs, depending on the solute–solvent interaction. They may alter the rate and mechanism of chemical reactions. Hence, play a significant role in the stabilization of pharmaceutical products.
As per ICH guidelines, class I solvents should be avoided in the manufacturing of the excipients, drug substance and drug products unless it is justified in a risk benefit assessment. Class II solvents should be limited in chemical synthesis and their acceptable limits range from 50 ppm to several thousand parts per million (ppm). Class III solvents are less toxic but however need to be quantified.

Requirement of fast GC method : While process development for a discovery molecule, multiple reactions are designed in such a way that finalized process will be cost effective & timeline oriented which enhances the productivity. Further, fast sample analysis is required to monitor the kinetics, formation of desired product and to make the scientific decision. Hence fast GC becomes an important technique for the drug discovery field.

Nexis GC-2030- A perfect tool:
While doing process development for a discovery molecule, multiple reactions are designed in such a way that the process will be affordable & timeline oriented which enhances the productivity.
Continuously running GC system may lead to high consumption of utilities, such as carrier gas, electricity etc. Active time management i.e automatic startup and shutdown function in the Nexis GC-2030 NX (Fig. 2) with LabSolutions software will help to save the time as well as analysis cost by minimizing utilities.
Unique features of Shimadzu's Nexis GC-2030 instrument like fast oven ramping, efficient AFC & APC, high inlet pressure, all flow control modes (flow, pressure & linear velocity), fast data acquisition rate, lower gas flow in the detector, sensitive FID detector make it as a perfect tool for the fast analysis.

Fig.2 Nexis™ GC-2030 NX with AOC™-20i

■ Experimental

During method development a mixture of 46 solvents were prepared in N-Methyl-2-Pyrrolidone and injected on various GC columns such as Rtx-5, Rtx-1301 & Rtx-624. However, optimum resolution and response was observed on Rtx-624 column with 20 m × 0.18 mm ×1 µm dimensions.

Furthermore, linearity was prepared from 10% to 140% considering ICH Q3C [1] limit as 100% for classified solvents (except benzene) and 5000 ppm for non-classified solvents. Precision was performed at 100% linearity level.

■ Method

Method details such as column. Instrument & analytical conditions are mentioned in Table 1.

Table 1 Analytical conditions and method parameters

GCMS System	**: Nexis GC-2030 with AOC™ -20i autosampler**
Column	: Rtx-624 20 m, 0.18 mm I.D., 1 µm df
Injection Volume	: 0.2 µL
Injector temperature	: 250 °C
Injection Mode	: Split
Split ratio	: 200
Carrier gas saver	: ON
High pressure injection	: 100 kPa for 1 min
Carrier Gas	: Helium
Flow control mode	: Linearity velocity
Linear Velocity	: 46.6 cm/sec
Purge Flow	: 3 mL/min
Temp. Program	: 42 °C (1.5 min), 12 °C/min to 72 °C (0.5 min), 80 °C/min to 240 °C (0.4 min).
Detector	: FID
Detector temperature	: 250 °C
Diluent	: NMP (N-Methyl-2-Pyrrolidone)

■ Linearity Solutions

Linearity Standard solutions of all solvents were prepared as mentioned in Table 2. Triplicate injections of each level were performed and 100% level was injected in the six replicate to check system precision.

Table 2 Linearity standard solution preparations

Linearity Levels	Volume from Linearity stock solution (mL)	Final diluted volume (mL)	% conc. w.r.to ICH-Q3C limit
Level-1	0.10	10	10
Level-2	0.50	10	30
Level-3	0.75	10	50
Level-4	1.00	10	100
Level-5	1.40	10	140

**Linearity stock concentration for all solvents was 10x to the limit specified in ICH-Q3C guideline.*

Fig. 3 to 8 depict the calibration curves, & level-1 chromatograms for representative solvents such as Acetaldehyde, Tertiary Butyl methyl ether, Methyl Cyclopentanone, Heptane, 1-Pentanol & Ethyl Benzene.

Acetaldehyde

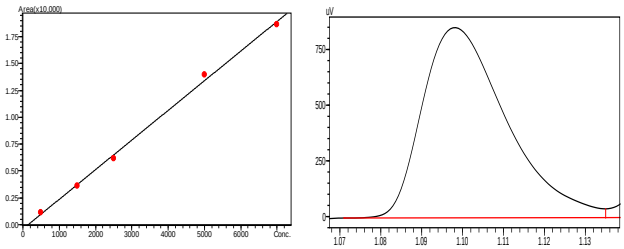

Fig.3 Calibration curve and reference chromatogram of Linearity level-1 solution for Acetaldehyde

Tertiary butyl methyl ether

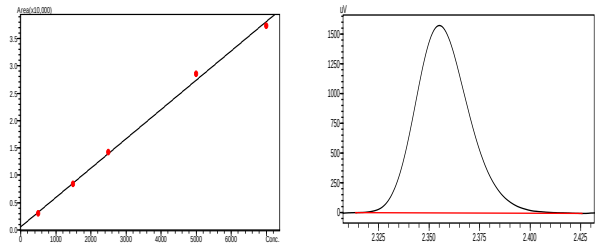

Fig. 4 Calibration curve and reference chromatogram of Linearity level-1 solution for Tertiary butyl methyl ether

Methyl Cyclopentanone

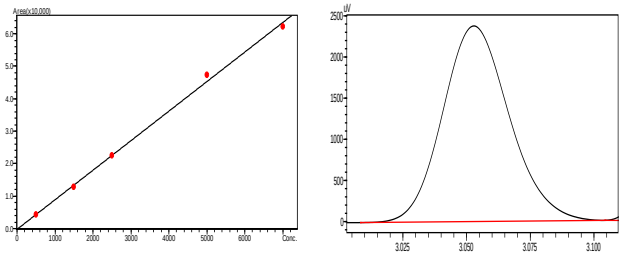

Fig. 5 Calibration curve and reference chromatogram of Linearity level-1 solution for Methyl Cyclopentanone

Heptane

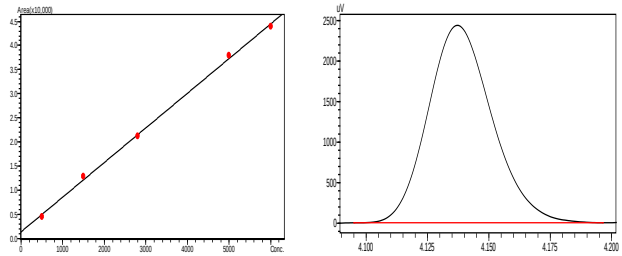

Fig. 6 Calibration curve and reference chromatogram of Linearity level-1 solution for Heptane

1-Pentanol

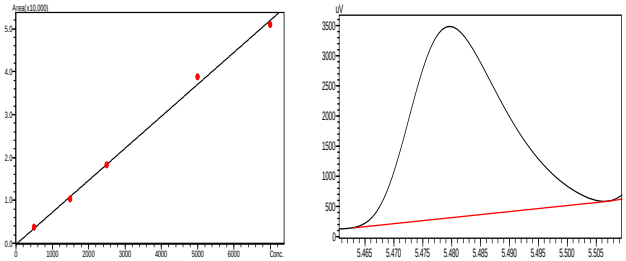

Fig. 7 Calibration curve and reference chromatogram of Linearity level-1 solution for 1-Pentanol

Ethyl Benzene

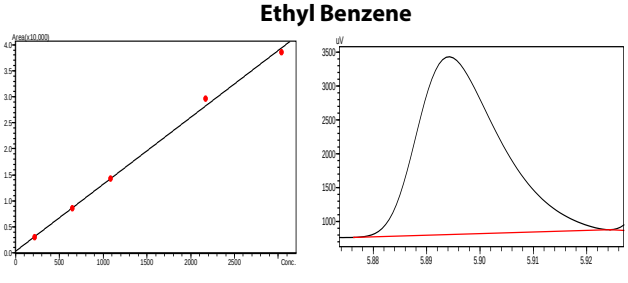

Fig. 8 Calibration curve and reference chromatogram of Linearity level-1 solution for Ethyl Benzene

■ Results

Five-point linearity standards were injected in triplicate injections. System Precision was performed at linearity level-4 by injecting six replicates and %RSD of area observed was evaluated. It was found well within the acceptance criteria as mentioned in ICH Q3C (i.e NMT 15 %).

■ Results (Cont).

Also evaluated the %RSD for retention time, resolution, co-relation coefficient, signal to noise etc. Most of the solvents are well separated. However, few solvents showing critical separation are reported in Table 4. Results for the study are summarized in Table 3. Reference chromatogram for linearity level solution-4 is marked in Fig. 9.

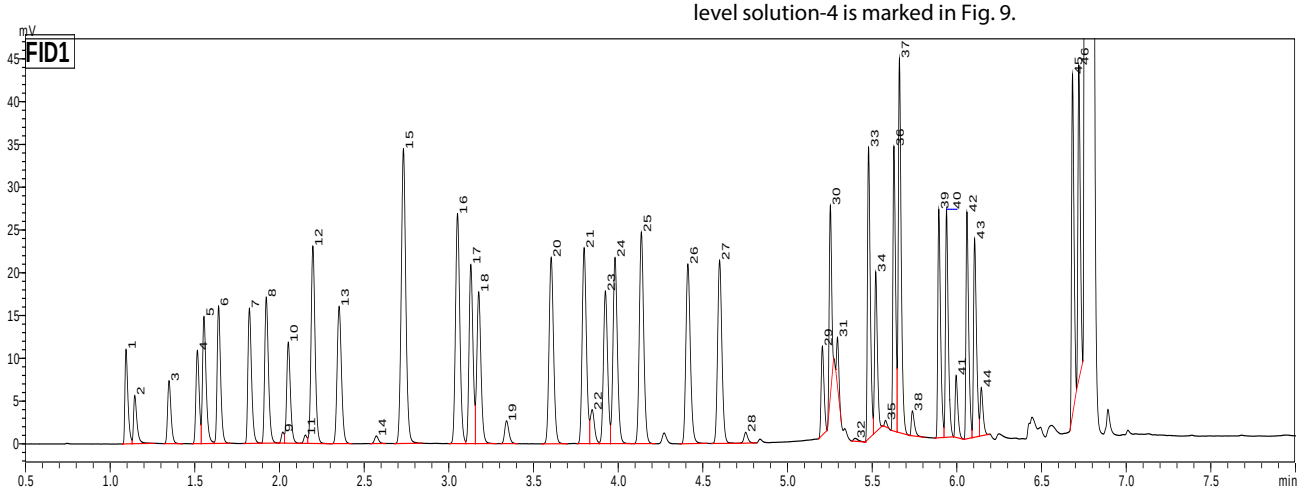

Fig. 9 Reference chromatogram of Linearity level-4 standard injection.

Table 3 Summarized results

Peak ID	Name of the solvent	RT (Min)	Conc. of Linearity Level-4 (ppm)	Linearity Range (ppm)	%RSD of area at Linearity Level-4	S/N at Linearity Level-1	Resolution	Correlation coefficient
1	Acetaldehyde	1.1	5000	500-7000	3.3	47	--	0.997
2	Methanol	1.15	3000	300-4200	1.7	23	1.4	0.998
3	Isopentane	1.35	5000	500-7000	1.5	27	4.7	0.998
4	Pentane	1.52	5000	500-7000	1.8	42	4.2	0.998
5	Ethanol	1.56	5000	500-7000	1.3	58	1.1	0.999
6	Diethyl Ether	1.64	5000	500-7000	1.4	67	1.9	0.998
7	Acetone	1.83	5000	500-7000	1.3	70	4.4	0.998
8	IPA	1.93	5000	500-7000	1.3	76	2.3	0.998
9	Acetonitrile	2.03	410	41-574	1.9	6	0.6	0.998
10	Methyl Acetate	2.06	5000	500-7000	1.2	60	0.2	0.998
11	Dichloromethane	2.15	600	60-840	2.6	5	2.2	0.996
12	Cyclopentane	2.2	5000	500-7000	1.4	119	1	0.998
13	t-butyl methyl ether	2.36	5000	500-7000	1.4	87	3.3	0.998
14	Hexane	2.57	290	29-406	2.2	5	4.6	0.998
15	1-Propanol	2.74	5000	500-7000	1.4	166	3.1	0.998
16	Methyl Cyclopentane	3.05	5000	500-7000	1.4	132	6	0.998
17	Methyl Ethyl Ketone	3.13	5000	500-7000	1.3	104	1.7	0.998
18	Ethyl Acetate	3.18	5000	500-7000	1.5	86	1	0.997
19	THF	3.35	720	72-1008	1.2	14	3.4	0.998
20	Cyclohexane	3.61	3880	388-5432	1.4	110	5.3	0.998
21	Isobutanol	3.8	5000	500-7000	1.7	118	4	0.997
22	Benzene	3.85	500	50-700	1.6	23	0.8	0.997
23	Isopropyl Acetate	3.93	5000	500-5000	1.3	99	1.3	0.999
24	Methyl THF	3.99	5000	500-5000	1.5	119	1.2	0.999
25	Heptane	4.14	5000	500-5000	1.5	135	3.2	0.999
26	1- Butanol	4.42	5000	500-5000	1.6	110	5.6	0.999
27	t-Butyl Acetate	4.6	5000	500-5000	1.5	121	3.7	0.999
28	1,4- Dioxane	4.76	380	38-400	2.8	7	3.5	0.998
29	Cyclopentyl Methyl ether	5.21	1500	150-2100	1.4	59	12.3	0.998
30	Isoamyl Alcohol	5.25	5000	500-5000	1.3	120	1.6	0.999
31	Toluene	5.3	890	89-1400	2.6	33	1.6	0.997
32	Ethylene glycol	5.44	1860	186-868	9.6	10	4.5	0.959
33	1-Pentanol	5.48	5000	500-7000	1.6	176	1.3	0.998

Continued…

Peak ID	Name of the solvent	RT (Min)	Conc. of Linearity Level-4 (ppm)	Linearity Range (ppm)	%RSD of area at Linearity Level-4	S/N at Linearity Level-1	Resolution	Correlation coefficient
34	Tertrachloroethylene	5.52	5000	500-7000	1.6	81	1.5	0.999
34	2-Hexanone	5.58	50	15-70	2.8	14	2	0.991
36	n-Butyl acetate	5.63	5000	500-7000	1.4	174	4	0.998
37	Cyclopentanone	5.66	5000	500-7000	1.8	229	1.1	0.998
38	DMF	5.75	880	88-1232	1.4	12	2.6	0.998
39	Ethyl Benzene	5.89	2170	217-3038	1.7	145	4.6	0.998
40	p-Xylene	5.94	2170	217-3038	1.6	144	1.5	0.997
41	4-Heptanone	6.00	1000	100-1400	1.6	41	1.9	0.998
42	o-Xylene	6.06	2170	217-3038	1.6	149	2.2	0.998
43	DMSO	6.12	5000	500-7000	1.6	87	1.7	0.998
44	DMAC	6.15	1090	109-1526	1.9	33	0.8	0.998
45	1-Octanol	6.69	5000	500-7000	2.9	218	16	0.998
46	Benzyl Alcohol	6.73	5000	500-7000	3.5	137	1.6	0.997

Note: Solvents which are not classified under ICH Q3C, are considered to control at 5000 ppm.

■ Critical Pairs

During method optimization few of the pairs were found critical to separate in fast GC technique. Resolution between these pair can be achieved under a condition in which the solvents eluting closer to the critical solvent pair shall not be the analytes of interest.

During actual analysis if all solvents are not included then resolution between this pairs can be improved.

Table 4 Critical solvent pairs

Solvent Pair	Resolution
Acetonitrile & Methyl Acetate	0.2
Iso-butanol & Benzene	0.8
DMSO & DMAC	0.8

■ System Suitability

ICH Q2 (R2) [2] was referred to design the experiments. System suitability criteria is applicable for analysis of finished drug products. However, in case of drug discovery sample, those criteria are eased to maximize the sample output and minimize the analysis time.

System suitability observed in this analysis is as per mentioned below,

- Correlation coefficient for all the solvents which are analyzed in this method, are more than 0.99.
- Relative standard deviation of area response observed during six injections of linearity level-4 for all solvents was found to be below 10%.
- Resolution for all solvent except critical pairs was observed between 1.0 to 6.0.

■ Conclusion

- 46 commonly used solvents in drug discovery process were successfully analyzed in single method with very short run time using Shimadzu's Nexis GC-2030.
- Nexis GC-2030 is a perfect tool for drug discovery applications due to it's salient features like fast oven ramping, highly sensitive FID detector and active time management software function.

■ References

1. ICH Q3C(R8): Guideline for residual solvents
2. ICH Q2 (R2): Guideline for analytical method validation

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