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Comprehensive GCMS-HS screening method for the extractables present in various pharmaceutical packaging materials

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Abstract
The identification and control of volatile and semi-volatile extractable compounds originating from pharmaceutical packaging materials is paramount for drug safety and regulatory compliance.[1] These compounds can potentially migrate into the drug product and compromise patient health. Conventional analytical methods are often challenged by the complexity of the volatile profiles released from polymeric packaging materials.[2] This paper presents the development and validation of an optimized Headspace Gas Chromatography–Mass Spectrometry (GC-MS/HS) screening method.[2] GC-MS is the established technique for volatile analysis, leveraging its high sensitivity and mass spectral library matching capabilities for the non-targeted identification of unknown extractables.[4] The method is optimized using static headspace with high-temperature extraction suitable for packaging samples, followed by highly selective single-column chromatographic separation.[1] Validation, executed according to ICH Q2(R1) guidelines [8], confirms the high specificity required to differentiate target extractables from matrix components, with expected accuracy (recovery) of 80.0%–120.0% and precision (%RSD) below 15%.[9] This method establishes a robust and selective analytical tool for the comprehensive screening and analysis of volatile extractables in pharmaceutical packaging materials.

Keywords: GC-MS, Headspace, Extractables, Leachables, Packaging, VOCs, Screening

Introduction
The chemical integrity of a pharmaceutical product is fundamentally dependent on the packaging system used for storage [1]. Volatile Organic Compounds (VOCs) and semi-volatile extractables, which may originate as polymer additives, manufacturing residues, or degradation products within packaging materials, pose a significant risk if they migrate (leach) into the drug product. Regulatory frameworks require rigorous testing—known as Extractables and Leachables (E&L) studies—to identify and quantify these potential contaminants and ensure patient safety [1]

Headspace Gas Chromatography–Mass Spectrometry (HS-GC-MS) is the industry-standard technique for analyzing volatile and semi-volatile components in pharmaceutical samples, including residual solvents and packaging extractables [2]. The technique offers excellent sensitivity and the distinct advantage of Mass Spectrometry (MS) detection, which allows for structural elucidation and confirmation of identity via mass spectral library searching, crucial for non-targeted screening studies of complex extractable profiles [4]. While highly complex extracts may present challenges in single-column separation, the specificity gained through MS detection remains invaluable for initial screening and targeted analysis [5].

The developed HS-GC-MS method described herein is optimized explicitly for high-temperature static headspace sampling of packaging materials, providing a highly sensitive and selective approach for characterizing the volatile extractable profile, thereby supporting regulatory compliance efforts.

Experimental Section

Materials

USP Residual Solvent Class-2 Mix-A ampoules and Dimethyl sulfoxide (DMSO) were purchased from Sigma Aldrich. Empty non-PVC infusion bags (100 mL) were bought from Medical Supply Stores.

Sodium chloride, potassium dihydrogen phosphate, dibasic phosphate, sodium phosphate monobasic and sodium phosphate dibasic, and Sodium sulphate anhydrous were of chemical grade.

Generating Extract

The extractables study was performed with the following extraction solutions to cover the wide pH range of the products with Infusion bags.

1. pH 3 Phosphate Buffer
2. 0.9 % Saline Buffer
3. pH 8.5 Phosphate Buffer

Infusion bags are filled with all three buffer solutions (Acidic, Neutral, and Basic). This configuration creates a worst-case surface area exposure of the bag to the different pH extraction solvents, resulting in a more concentrated extract from the bag. Controls were filled in borosilicate glass bottles for organic extractable analysis. Filled bags and controls were autoclaved (Make: Labindia, Model L-SAA 50) at 121 ˚C for 30 mins and then stored at 50 ˚C for 30 days.

After aging, the bags were stored at 2-8 °C. Before analysis, the bags were allowed to reach ambient temperature and were then cut and used. For each sample preparation, the required amount of extract was directly taken from the individual bag. The same procedure was followed for all three extraction solutions.

System Suitability Standard

Stock Solution: Transfer 1.0mL of the USP Residual Solvent Class 2 Mix-A into a 100mL volumetric flask and dilute to volume with DMSO.

Spiking Solution: Transfer 10mL of the stock solution to another 100mL volumetric flask and dilute to volume with DMSO.

Working solution: Accurately weigh approximately 2 grams of sodium sulfate in a headspace vial. Pipette 1.0mL of the system suitability spiking solution into the headspace vial and 4 mL of water, and crimp the vial.

Sample Preparation

Samples were prepared by transferring 4.0 mL of each extract sample (pH 3, pH 8.5, and 0.9% Saline) into a headspace vial containing 2.0 g of sodium sulfate and 1 mL of DMSO. Vials were crimped and closed. Samples were analysed along with the system suitability solution.

Control Preparation

Control samples were prepared by transferring 4.0 mL of each control extract (pH 3, pH 8.5, and 0.9% Saline) into a headspace vial containing 2.0 g of sodium sulfate and 1 mL of DMSO. Vials were crimped and closed.

Instrumentation

Headspace Instrumentation

Headspace (HS) sampling is employed for sample introduction due to its suitability for characterizing volatile components in solid matrices, such as packaging materials, using simple pretreatment.[7] High-temperature extraction via the headspace sampler (Thermo Fisher Scientific Triplus 300) is used to efficiently drive extractables from the polymer matrix into the vial headspace for subsequent analysis [1].

Headspace Conditions

Headspace Conditions	
Oven temperature	85˚C
Loop temperature	95˚C
Transfer line temperature	105˚C
Vial equilibration	30 minutes
Injection duration	0.50 minute
GC cycle time	40 minutes
Vial size	20 mL
Vial shaking	Medium
Loop Fill mode	Pressure
Injection mode	Standard
Loop equilibration time	0.5
Loop final pressure	50 psi
Auxiliary Pressure (Vial)	100 psi

GC-MS Instrumentation

The analysis is performed using a Gas Chromatography (GC) system coupled to a Mass Spectrometer (MSD) (Make: Thermo Scientific ISQ 7610 single quadrupole GC-MS system, trace 1300) for highly selective detection and identification. This setup is specifically chosen for screening complex volatile profiles.

GC Column and Conditions

The method employs a single capillary column typical for volatile analysis, balancing resolution with run time for efficient screening

	Rate (˚C/min)	Temp (˚C)	Hold Time (min)
Oven program	-	40˚C	-
	10	100	10
	15	220	10
Column	DB-624 (60m x 0.25mm x 1.4um)		
Split flow	10 mL/min		
Mode	Constant Pressure		
Injection temperature	155˚C		
Purge flow	5 mL/min		
Column Inlet Pressure	250 kPa		
Detector Temperature	250 ˚C		
Air Flow	400 mL/min		
Hydrogen	45 mL/min		
Make up gas flow	25 mL/min		

Mass Spectrometry (MS) Detection

Compound identification and quantification were performed using a Mass Spectrometer (MSD) [7]. The MS detector provides high selectivity via fragmentation patterns, which is essential for accurate identification of unknown extractables using mass spectral libraries (e.g., NIST library) [4].

MSD Conditions

Parameter	Default Setting
Transfer Line Temperature	220 ˚C
Ion Source Temperature	200 ˚C
Electron Energy	70 eV (Electron Ionization, EI)
Acquisition Mode	Full scan (m/z 35–400)

Volatile organic extractables analysis was performed using Headspace Gas Chromatography with Flame Ionization Detection and Mass Spectrometry (HSGC-FID/MS).

The concentration of the volatile extractables was estimated using a semi-quantitative method, based on the average area of all system suitability mix compounds at a concentration of 1 ppm. The linearity of the process was established over the range of 0.05 µg/mL to 40 µg/mL, and method suitability was demonstrated by performing accuracy checks across this linearity.

The control and extracts were compared. Peaks that were not present in the control were reported and identified by HSGC-FID/MS.

The definitions of the compound identification levels are presented below:

- Tentative: Structure identified with GCMS data and /or NIST Library match (Below 80%).
- Confident: Structure identified with NIST Library match (Above 90 %) and MS spectral match
- Confirmed: Structure identified with an authentic reference compound

Data Processing

The MS data is processed using Chromeleon chromatographic software. Automated peak deconvolution, retention time alignment, and accurate library searching (e.g., NIST library) are essential tools for non-target screening to identify unknown extractables. Chemometric methods can be employed for comparative analysis across different packaging batches or materials.

Results and Discussion

Chromatographic Performance and Specificity

The developed HS-GC-MS method demonstrated high specificity, crucial for E&L screening.[16] The method successfully extracted and separated numerous volatile organic compounds from pharmaceutical packaging types [1] Chromatographic performance showed reproducible and clear separation of key volatile markers, with MS detection providing definitive compound identification via spectral matching. The use of MS ensures that potential co-eluting peaks are differentiated through mass spectral fingerprints, confirming the method's specificity for accurate screening [4].

HSGC-FID chromatogram of System Suitability solution

System suitability chromatogram **Fig 1** demonstrates that all the peaks of various chemical natures and boiling points are separated well. The Details are provided in Table 1. This indicates that the method is capable of eluting the different categories of volatile compounds, which may leach into the drug product from the pharmaceutical packaging materials.

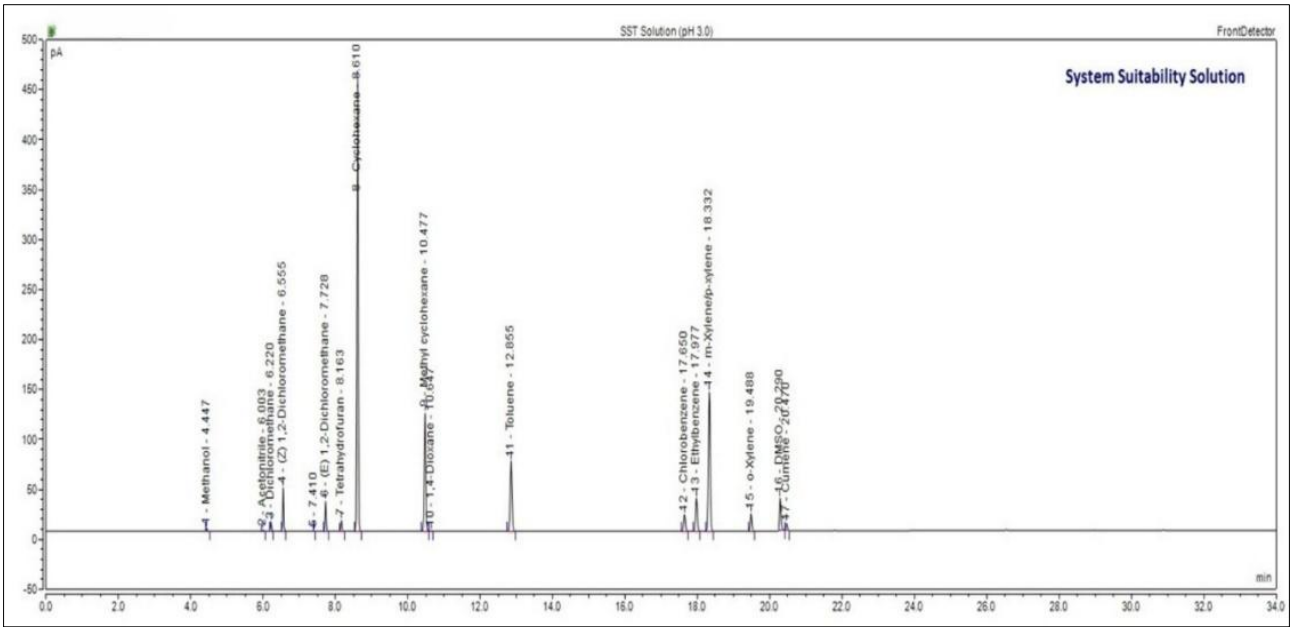

Fig 1: System Suitability Chromatogram

HSGC-FID chromatogram of the Sample solution Extract

Sample solution extract pH 3.0 Buffer Solutions-Control, Day 0 (T0 Sample), and Day 30 (T30) were injected into the GC-MS. Their chromatograms were overlaid (Fig 2) and compared with the control. Peaks observed at the same RT in the control sample were not reported**.** Similarly, the processing pattern was applied for the sample extract, 0.9 %Saline solution, and pH 8.5 Buffer solution, and their chromatography was compared (Fig 3 and Fig 4). The content of each observed peak in the respective samples (Control, Day 0, and Day 30) was determined by comparison with the standard solution. Mass spectra of each peak were obtained, and the NIST Library was used to compare the library match. Compounds are those with a % NIST match found above 90% and matched with the respective standard, categorized as confirmed (Table 2).

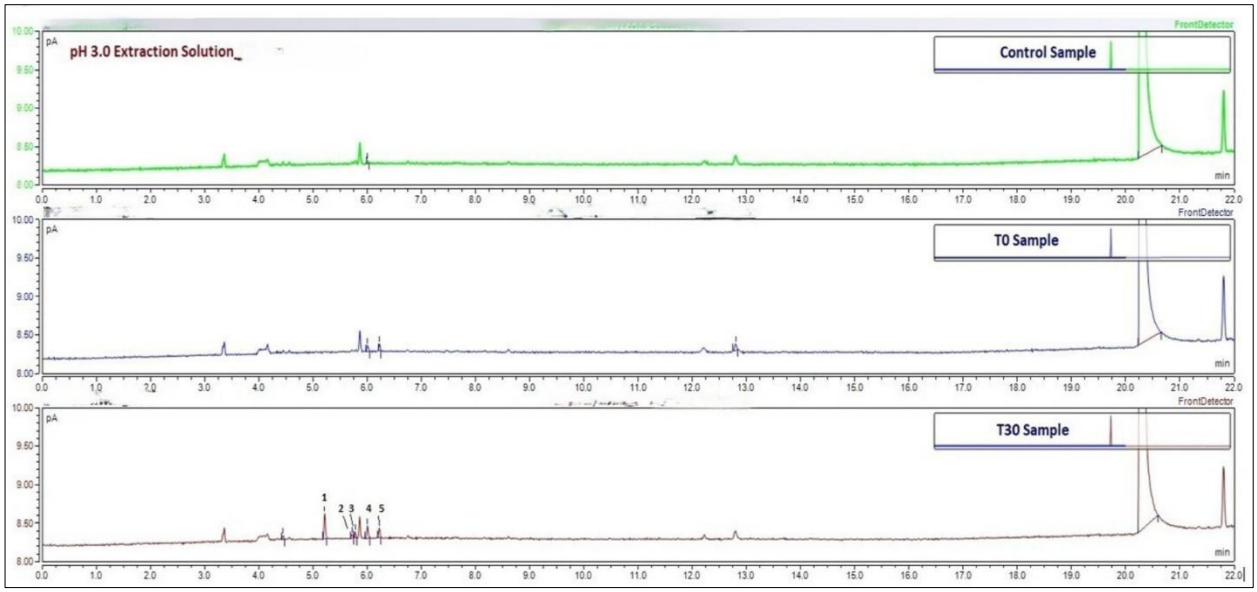

Fig 2: Overlay chromatogram of Sample solution, Extract pH 3 Buffer solution

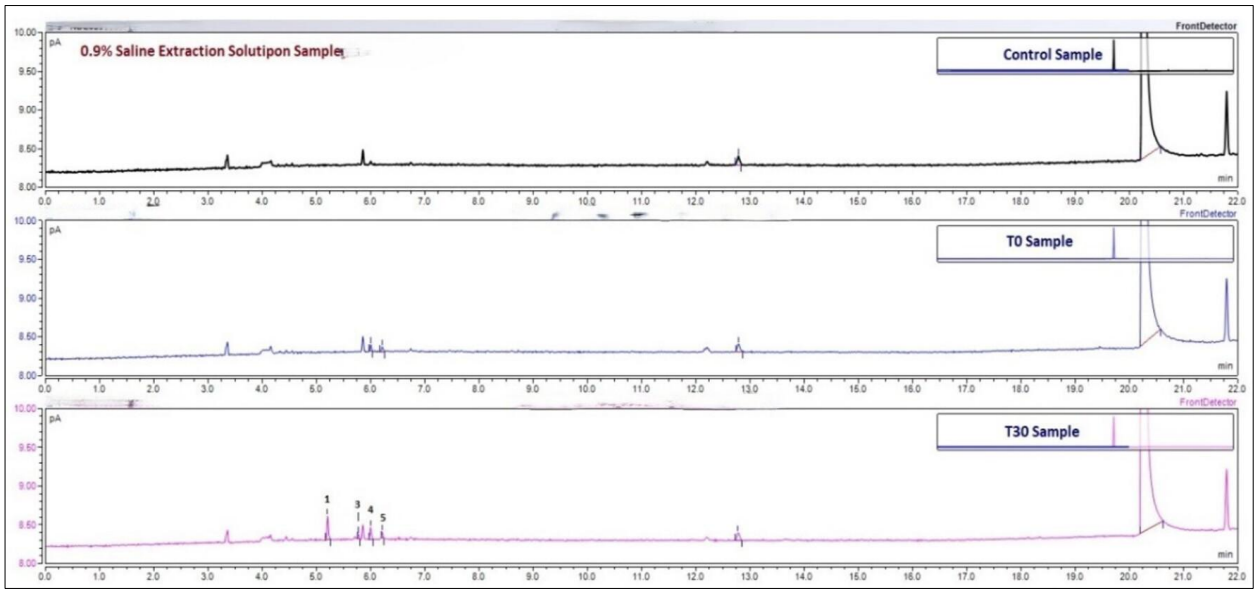

Fig 3: Overlay chromatogram of Sample solution, Extract 0.9 % Saline Buffer solution

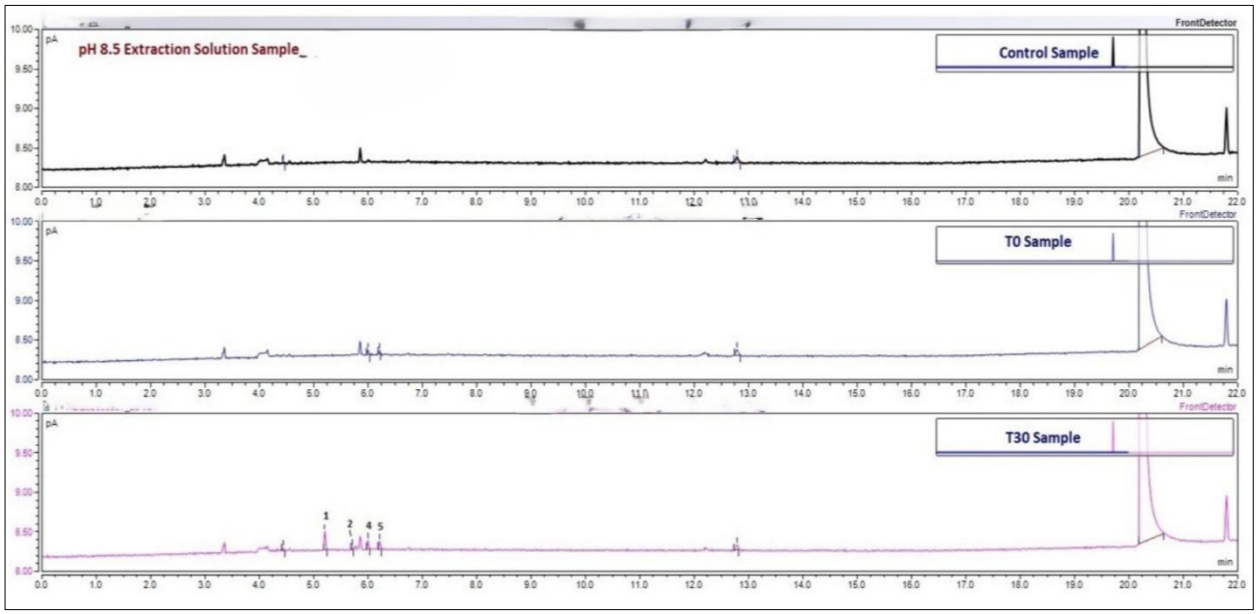

Fig 4: Overlay chromatogram of Sample solution, Extract pH 8.5 Buffer solution

Table 1: Details of Volatile Organic Compounds with Retention Time and Boiling Point

Compound	CAS Number	Boiling Point	RT (min)
Methanol	67-56-1	64.7	4.5
Acetonitrile	75-05-8	81.6	6.0
Dichloromethane	75-09-2	39.6	6.2
(Z) 1,2-Dichloromethene	156-59-2	60	6.6
(E) 1,2-Dichloromethene	156-60-5	47-49	7.7
Tetrahydrofuran	109-99-9	66	8.2
Cyclohexane	110-82-7	80.7	8.6
Methyl cyclohexane	108-87-2	101	10.5
1,4-Dioxane	123-91-1	101-102	10.6
Toluene	108-88-3	110.6	12.9
Chlorobenzene	108-90-7	131	17.7
Ethylbenzene	100-41-4	136	18.0
m-Xylene/p-Xylene	108-38-3	139	18.3
o-Xylene	106-42-3	138	19.5
DMSO	95-47-6	144	20.3
Cumene	67-68-5	189	20.5

Table 2: Details of Volatile Organic Compounds with observed concentration

RT (min)	Peak No	Extractable	Identification Level	Estimated Concentration (µg/mL)					
				pH 3.0		pH 8.5		0.9% Saline	
				T0	T30	T0	T30	T0	T30
5.21	1	Ethanol	Confirmed	0.000	0.015	0.000	0.012	0.000	0.014
5.72	2	Acetone	Confirmed	0.000	0.003	0.000	0.002	0.000	0.000
5.78	3	Isopropyl alcohol	Confirmed	0.001	0.003	0.000	0.000	0.000	0.002
6.00	4	Acetonitrile	Confirmed	0.002	0.008	0.004	0.005	0.004	0.007
6.22	5	tert-Butanol	Confirmed	0.003	0.006	0.003	0.005	0.003	0.004

Conclusion

The Headspace Gas Chromatography–Mass Spectrometry (GC-MS/HS) method developed herein represents a highly selective and sensitive analytical strategy for characterizing the volatile extractable profiles of pharmaceutical packaging materials. By leveraging the inherent specificity and sensitivity of MS detection, this method provides reliable identification and quantification for complex sample analysis.[4] The method was able to detect and resolve diverse categories of volatile organic compounds, confirming its reliability as a robust analytical tool, making it invaluable for comprehensive quality control screening and non-targeted discovery work within the pharmaceutical packaging industry [9].

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