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METHOD DEVELOPMENT AND VALIDATION FOR QUANTIFICATION OF SEMI-VOLATILE ORGANIC COMPOUNDS IN SUMATRIPTAN INJECTION USING GC-MS/MS

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ABSTRACT

Single-use systems are crucial in pharmaceutical manufacturing, storage, and packaging. Chemical additives used to enhance plasticity and elasticity in prefilled syringes could be detected as extractable and can potentially migrate to the finished formulation as leachable impurities, which are known to cause significant health hazards. The need to efficiently identify, quantify, and manage the risks related to the exposure of potentially leached compounds in the final formulation is of critical importance. The semi-volatile organic compounds are those that have boiling points between 240°C and 300°C. They can exist in both the gas phase and the particulate phase in a given system. These compounds are associated with reproductive toxicity, neurotoxicity, and carcinogenicity. Therefore, assessing the levels of semi-volatile organic compounds in parenteral formulations is a vital requirement. The current study intends to develop and validate an analytical method to identify and quantify Semi-Volatile Organic Compounds by using Gas Chromatography and Tandem Mass Spectrometry.

Keywords: Semi-Volatile Organic Compound, Extractable, Leachable, GC-MS/MS, Chromatography, Method development, Validation.

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INTRODUCTION

Prefilled syringes are one of the important single-use systems (SUSs) that are becoming prominent as container closures for packaging and storage of parenteral formulations.[1] However, chemical additives used to enhance material features such as elasticity and plasticity in SUSs can migrate as impurities into the final product.[2] Detection of extractable and leachables (EandL) during the drug development stage is critical to ensure patient safety and product quality.[3] To evaluate the hazardous influence of leachables on the drug products' suitability, the final formulation needs to be evaluated to discover, identify, and quantify potential leachables. This requires a multi-method analytical strategy due to their chemical diversity.[4-6] The criticality of extractable and leachable analysis is primarily based on the type of formulation, administration dose, and frequency.[7] Accordingly, there is a high potential for safety issues related to the administration route. It is often the case that the formulations intended for the parental route of administration could lead to maximum safety risks about leachable impurities.[8] Chemically, the compounds that are regarded as extractable and leachable can be categorized as follows: Semi-volatile organic compounds (SVOCs), volatile organic compounds (VOCs), non-volatile compounds (VOCs), and inorganic elemental compounds.[9] The compounds that are used as solvents, antioxidants, lubricants, plasticizers, and polymer degradants, will contribute to potential SVOCs. The semi-volatile organic compounds are those that have boiling points between 240°C and 300°C. These are the organic chemicals with vapor pressures between those of volatile organic compounds and non-volatile organic compounds. They can exist in both the gas phase and the particulate phase in a given system.[10] SVOCs include hydrocarbons, polycyclic aromatic hydrocarbons, ethers, phenols, ketones, aldehydes, organic acids, esters, amines, amides, polychlorinated biphenyls, phthalate esters, haloethers, trihalomethanes, nitrosamines etc. and can cause severe health issues such as

mucosal irritations at the nose and throat, ophthalmic irritation, nausea and vomiting, dyspnea, epistaxis, renal and hepatic dysfunction. These compounds are associated with reproductive toxicity, neurotoxicity, and carcinogenicity. In addition, they have been found in human biological fluids (blood and urine).[11] Gas chromatography is a widely used analytical technique for separating and detecting chemical components in sample mixtures.[12] Sumatriptan, a commonly used triptan for migraine treatment, is available in oral, intranasal, and subcutaneous formulations approved by the US FDA. As substantiated by a systematic Cochrane review, the subcutaneous delivery of sumatriptan is clinically superior over the oral and inhalational delivery formulations.[13] Considering the peripheral nervous system site of action of sumatriptan, which might render a chance for the development of neuronal toxicity from the presence of any potential SVOC leachable compounds in the finished parenteral formulation, Sumatriptan Injection [Auto Injector] at a higher dose, i.e 6 mg/0.5 mL, was selected for the study to develop and validate an analytical method as per Fig.-1 to determine the SVOCs by using Gas Chromatography Tandem Mass Spectrometry (GC-MS/MS). [14]

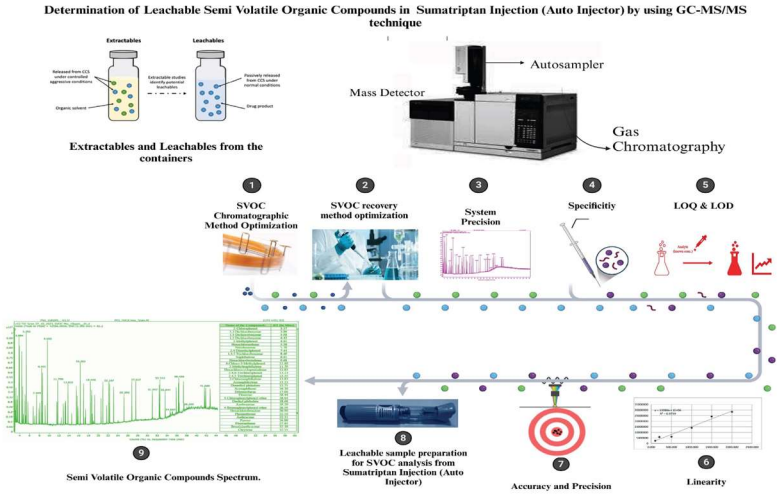

Fig.-1: Graphical Abstract

EXPERIMENTAL

Materials and Methods

Working standard Environmental Protection Agency Contract Laboratory Program (EPA CLP) SVOC mix, (Sigma Aldrich). High-performance liquid chromatography grade Dichloromethane (Rankem), Sodium Sulphate AR at ACS grade, and purified water. Instruments used were GC-MS instrument (Agilent 7890B GC System) with 7000D GC/MS Triple QUAD and Autosampler (Agilent 7693), Semi Micro Balance (radwag), refrigerator, freezer, water purification system, fume hood. DB-WAX, HP-Wax, and HP-5-MS columns were initially selected for analysis based on good peak symmetry and sensitivity for analysis of basic compounds that are highly polar, and also, based on literature recommendation, the HP-5-MS column has been selected for analysis of SVOCs such as PAHs and PCBs.[15]

General Procedure

The method was developed using the following standard EPA CLP SVOC mix, which necessitates generating a calibration curve and system suitability solution to determine SVOC concentrations in drug samples.[16] Hence, a mixture comprising compounds (EPA CLP SVOC Mix) with varying boiling points was injected, and all the compounds were eluted in this method. The AET (Analytical Evaluation Threshold) is developed using SCT (Safety Concern Threshold) for leachable profiles of specific drug products. As per the guidance, a SCT of 1.5 µg / day should be used to calculate the AET for aqueous-based parenteral product formulation.

Analytical Evaluation Threshold (AET) calculation of Sumatriptan Injection USP (6 mg/0.5 mL)

Based on the SCT of 1.5 µg / day, the AET which is also the LOQ (Limit of Quantification) was calculated

as 1.5 µg/mL (1500 ppb). The calculation was based on the maximum daily dose of sumatriptan, and the unit fill volume of the formulation being evaluated. In addition, an uncertainty factor of 0.5 was considered to refine the estimated AET as 0.75 µg/mL or ppm (750 ppb).

Preparation of Standards and Stock Solution

Standards and stock solutions required for the study were prepared using the EPA CLP SVOC mix. Which was further used to prepare linearity standards. Sumatriptan injection USP and EPA CLP SVOC Mix stock solutions were used to prepare specificity samples and LOQ accuracy samples.

RESULTS AND DISCUSSION

For measuring the presence of semi-volatile organic compounds in parenteral formulations like Sumatriptan Injection USP (6 mg/0.5 mL), the limit of quantification is 0.400 ppm using a GC-MS/MS instrument, and an HP-5-MS column was utilized. The method was developed for a mixture comprising of compounds (EPA CLP SVOC Mix) with varying boiling points ranging from 60°C to 300°C programmed ramp-up rate of 5°C/min that was injected into the carrier gas. All the compounds were eluted in this method. Nitrogen (N_2) gas at the flow rate of 1.5 mL/min was employed as collision gas, and Helium gas (He) gas at the flow rate of 2.25 mL/min was used as quench gas. The volume of Injection is 1.0 µL, M/Z Range scan is done from a range of 45-550 mass transition through MS1 SCAN. System precision, Specificity, Linearity Range, LOQ and LOD results are displayed in Table-1.

Table-1: System Precision, Specificity, Linearity Range, LOQ, and LOD

S. No	SVOC Compound Name	System Precision		Specificit-y (% Interferen-ce) (not more than 10 % with LOQ)	Linearity Range: 0.100 ppm - 2.000 ppm			LOQ Average S/N Ratio (not less than 10)	LOD (S/N Ratio) (not less than 3)
		Peak Area RSD (not more than 20 %)	Retention time RSD (not more than 5 %)		Co-relation coefficient (not less than 0.95)	Slope	Intercep-t		
1	2-CHLOROPHENOL	4.39	0.00	Nil	0.9759	13980	1000000	17.700	7.4
2	1,1,3 DICHLOROBENZENE	3.15	0.00	Nil	0.9819	14212	867811	18.000	9.6
3	1,4 DICHLOROBENZENE	6.08	0.01	Nil	0.9810	13556	1000000	16.967	9.1
4	1,2 DICHLOROBENZENE	5.27	0.09	Nil	0.9804	16145	1000000	14.700	7.1
5	2-METHYLPHENOL	5.32	0.00	Nil	0.9826	27945	2000000	22.700	10.2
6	HEXACHLOROETHENE	12.46	0.01	Nil	0.9804	8012.5	552926	9.867	6.5
7	NITROBENZENE	2.85	0.07	Nil	0.9669	9195.6	914937	12.667	4.5
8	2,4 DIMETHYLPHENOL	4.68	0.01	Nil	0.9872	11494	72686	10.800	4.1
9	1,3,5 TRICHLOROBENZENE	2.88	0.01	Nil	0.9856	18174	1000000	23.733	13.1
10	NAPHTHALENE	4.84	0.00	Nil	0.9826	9467.1	855892	9.800	4.9
11	HEXACHLOROBUTADIEN E	3.55	0.00	Nil	0.9780	13914	1000000	18.933	9.1
12	4-CHLORO-METHYLPHENOL	4.20	0.00	Nil	0.9746	10435	533287	8.600	5.5
13	2-METHYLNAPHTHALENE	3.98	0.01	Nil	0.9781	21746	1000000	26.833	11.8
14	HEXACHLOROCYCLOPEN T ADIENE	7.28	0.06	Nil	0.9793	14777	126855	10.600	5.5
15	2,4,6-TRICHLOROPHENOL	9.35	0.00	Nil	0.9575	10055	31307	7.267	3.7
16	2,4,5-TRICHLOROPHENOL	7.42	0.00	Nil	0.9656	10274	642597	4.900	ND
17	2-CHLORONAPHTHALENE	1.47	0.00	Nil	0.9765	20809	1000000	23.100	12.7
18	ACENAPHTHYLENE	4.39	0.01	Nil	0.9788	20743	1000000	22.467	10.4
19	DIMETHYL PHTHALATE	4.32	0.01	Nil	0.9809	16511	894187	19.200	8.4
20	ACENAPHTHENE	3.84	0.00	Nil	0.9760	32136	2000000	29.867	10.4
21	DIBENZOFURAN	3.20	0.00	Nil	0.9811	22280	2000000	25.333	11.9
22	FLUORENE	3.66	0.04	Nil	0.9788	28185	1000000	30.133	13.8
23	4-CHLOROPHENYLPHENYL ETHER	3.95	0.00	Nil	0.9735	41416	575691	21.833	18.6

24	AZOBENZENE	6.06	0.00	Nil	0.9356	16860	157217	15.667	8.7
25	4-BROMOPHENYLPHENYL ETHER	2.70	0.00	Nil	0.9743	13846	1000000	16.300	8.0
26	HEXACHLOROBENZENE	4.43	0.00	Nil	0.9818	14213	1000000	17.633	8.6
27	PHENANTHRENE	3.52	0.00	Nil	0.9826	30509	2000000	30.567	15.1
28	ANTHRACENE	4.83	0.00	Nil	0.9780	35172	1000000	29.333	13.5
29	PYRENE	5.15	0.00	Nil	0.9828	29936	2000000	29.400	15.2
30	FLUOROTHENE	6.18	0.00	Nil	0.9773	30343	3000000	26.233	14.4
31	BENZ(A)ANTHRACENE	5.44	0.00	Nil	0.9797	23780	2000000	23.167	9.6
32	CHRYSENE	5.24	0.02	Nil	0.9778	25746	2000000	22.067	10.6

Linearity

The Linearity of all the SVOC mix standard compounds eluted showed a correlation coefficient (r^2) greater than 0.95, except Azobenzene, i.e., 0.9356.

Limit Of Quantification (LOQ)

LOQ of all the SVOC mix standard compounds showed an average S/N ratio not less than 10, except Hexachloroethene: 9.867, Naphthalene: 9.800, 4-Chloro-Methylphenol: 8.600, 2,4,6-Trichlorophenol: 7.267, and 2,4,5-Trichlorophenol: 4.900.

Accuracy and precision

Accuracy and precision results are displayed in Table-2.

Average % accuracy at LOQ level (0.400 ppm) for all the SVOC mix standard compounds showed within 50% to 150%, except 1,1,3 Dichlorobenzene: 189.17%, 4-Bromophenylphenyl Ether: 298.963%, Fluorothene: 219.060%).

% RSD at LOQ level (0.400 ppm) for all the SVOC mix standard compounds showed not more than 30%.

Average % Accuracy at 50% concentration level (0.500 ppm) for all the SVOC mix standard compounds showed within 60% to 140%, except 1,1,3 Dichlorobenzene: 153.087%, Anthracene: 59.807% and Fluorothene: 168.193%).

Average % Accuracy at 100% concentration level (1.000 ppm) for all the SVOC mix standard compounds showed within 70% to 130%, except 2,4,6-Trichlorophenol: 64.048%.

Average % Accuracy at 150% concentration level (1.500 ppm) for all the SVOC mix standard compounds showed within 70% to 130%, except 2,4,6-Trichlorophenol: 67.943% and 4-chlorophenylphenyl ether: 69.867%.

% RSD at 50% concentration level (0.500 ppm) for all the SVOC mix standard compounds showed not more than 30%.

% RSD at 100% concentration level (1.000 ppm) for all the SVOC mix standard compounds showed not more than 30%.

% RSD at 150% concentration level (1.500 ppm) for all the SVOC mix standard compounds showed not more than 30%. (Except 4-Chloro-Methylphenol: 36.310 and 4- 4-Chlorophenylphenyl Ether: 33.390%).

The results for linearity, accuracy, precision, LOQ, and LOD all met the acceptance criteria. This approach is accurate, precise, and sensitive for detecting semi-volatile organic compounds in Sumatriptan Injection USP (6 mg/0.5 mL).

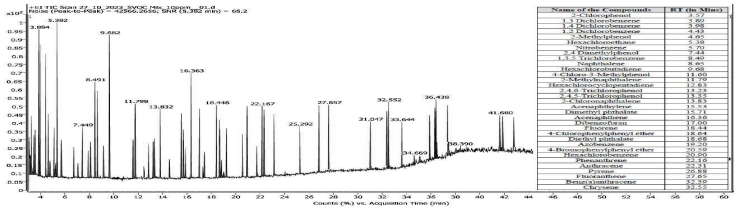

Fig.-2: Semi-Volatile Organic Compounds Mix Standard Spectra

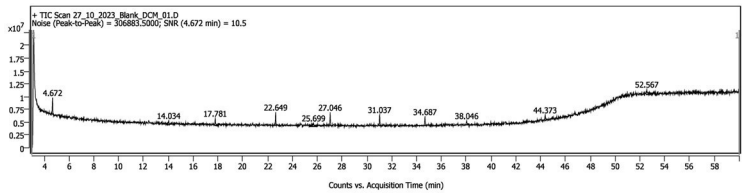

Fig.-3: Blank Chromatogram

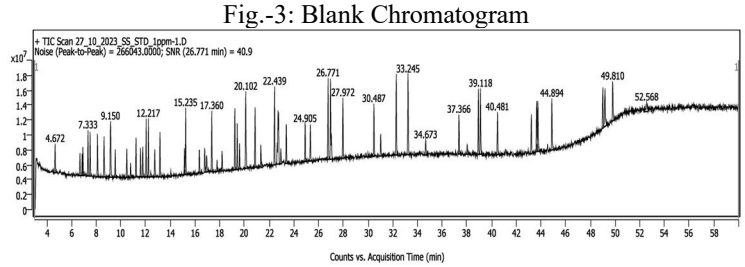

Fig.-4: System Suitability Standard Chromatogram

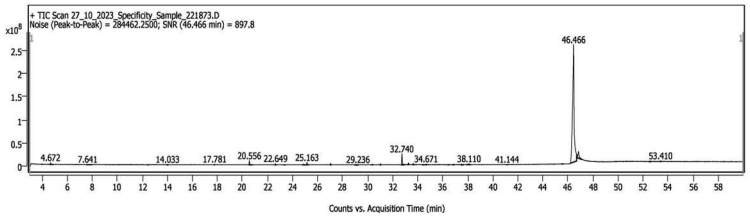

Fig.-5: Specificity Sample Chromatogram

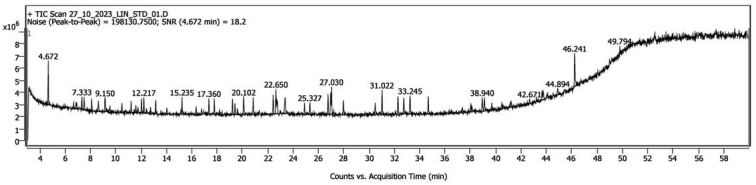

Fig.-6: Linearity Standard 01 Chromatogram

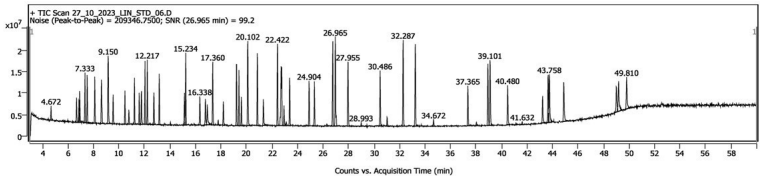

Fig.-7: Linearity Standard 06 Chromatogram

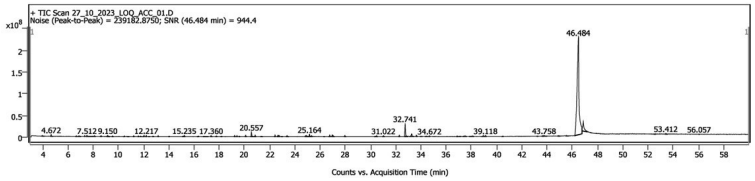

Fig.-8: LOQ Accuracy Chromatogram

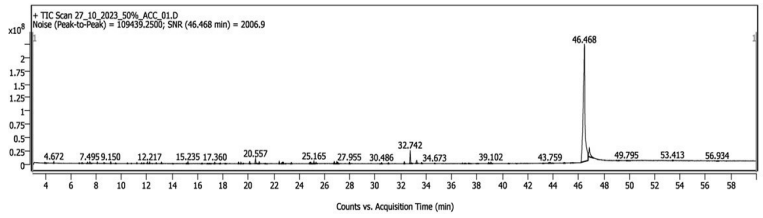

Fig.-9: 50% Accuracy Chromatogram

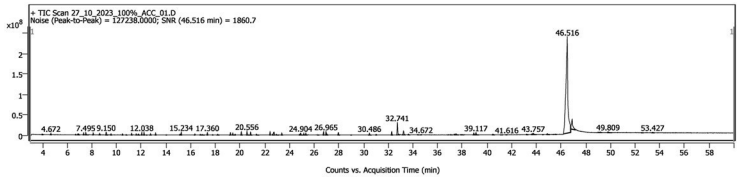

Fig.-10: 100% Accuracy Chromatogram

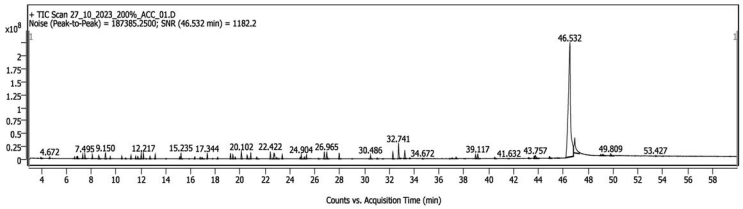

Fig.-11: 150% Accuracy Chromatogram

Table-2: Accuracy and Precision

S.No	SVOC Compound Name	Accuracy				Precision			
		Average % Accuracy at 50% concentration level (0.500 ppm) (Limit 60% to 140%)	Average % Accuracy at 100% concentration level (1.000 ppm) (Limit 70% to 130%)	Average % Accuracy at 150% concentration level (1.500 ppm) (Limit 70% to 130%)	Average % Accuracy at LOQ (0.400 ppm) (Limit 50% to 150%)	% RSD at 50% concentration level (0.500 ppm) (Not more than 30%)	% RSD at 100% concentration level (1.000 ppm) (Not more than 30%)	% RSD at 150% concentration level (1.500 ppm) (Not more than 30%)	% RSD (LOQ) (Not more than 30%)
1	2-CHLOROPHENOL	77.093	78.775	86.103	83.793	7.510	7.080	7.110	3.92
2	1,1,3 DICHLOROBENZENE	153.087	130.940	126.230	189.170	2.240	7.070	4.550	17.42
3	1,4 DICHLOROBENZENE	87.573	85.273	93.320	86.393	5.540	4.720	4.370	15.06
4	1,2 DICHLOROBENZENE	80.840	80.610	88.287	83.953	7.250	3.460	6.420	8.40
5	2-METHYLPHENOL	81.913	82.423	88.593	83.577	3.590	3.430	4.870	3.14
6	HEXACHLOROETHENE	81.507	77.643	82.837	83.460	8.750	7.070	4.490	8.10
7	NITROBENZENE	83.853	80.253	84.673	78.727	3.240	5.680	2.700	2.94
8	2,4 DIMETHYLPHENOL	75.367	78.290	84.470	71.860	13.010	6.010	2.770	7.69
9	1,3,5 TRICHLOROBENZENE	84.920	83.495	90.340	86.837	4.510	5.480	4.940	6.92
10	NAPHTHALENE	78.840	81.348	82.363	86.603	2.630	4.690	5.250	7.14
11	HEXACHLOROBUTADIENE	90.373	88.127	94.350	94.110	5.410	4.090	6.580	5.83
12	4-CHLORO-METHYLPHENOL	69.200	76.132	101.350	72.693	3.800	6.240	36.310	7.66
13	2-METHYLNAPHTHALENE	89.240	84.625	88.027	87.577	1.360	6.140	4.000	2.14
14	HEXACHLOROCYCLOPENTADIENE	83.087	78.288	79.993	89.800	5.180	3.290	4.790	16.72
15	2,4,6-TRICHLOROPHENOL	65.267	64.048	67.943	74.487	8.280	4.520	3.160	1.18
16	2,4,5-TRICHLOROPHENOL	78.500	79.965	90.490	74.537	4.370	12.690	8.480	9.48
17	2-CHLORONAPHTHALENE	85.033	82.928	88.513	85.570	0.730	3.530	3.320	3.40
18	ACENAPHTHYLENE	81.000	80.650	84.893	79.383	2.740	3.400	1.900	2.98
19	DIMETHYL PHTHALATE	79.940	79.248	83.430	78.567	0.710	3.140	1.430	1.92
20	ACENAPHTHENE	74.933	78.415	85.497	74.480	3.630	2.880	2.300	5.59
21	DIBENZOFURAN	94.473	87.990	93.650	102.713	3.300	3.700	3.850	7.34
22	FLUORENE	81.360	81.008	87.487	80.560	1.220	4.130	3.200	5.14
23	4-CHLOROPHENYLPHENYL ETHER	73.640	83.963	69.867	68.847	2.580	3.520	33.390	5.05
24	AZOBENZENE	84.313	82.553	88.707	101.637	4.010	4.180	4.090	23.61
25	4-BROMOPHENYLPHENYL ETHER	145.860	118.488	119.450	298.963	10.010	4.790	13.940	15.36
26	HEXACHLOROBENZENE	104.180	98.875	95.953	117.677	3.880	4.670	4.500	8.47
27	PHENANTHRENE	80.593	81.837	86.217	82.853	3.410	3.980	3.600	7.30
28	ANTHRACENE	59.807	73.537	76.833	61.177	6.730	13.230	2.340	2.76
29	PYRENE	75.420	81.108	85.783	75.993	4.590	4.500	1.860	4.11
30	FLUOROTHENE	168.193	125.267	110.047	219.060	12.150	6.650	4.180	2.68
31	BENZ(A)ANTHRACENE	68.213	71.185	76.620	75.153	12.610	6.160	2.760	3.49
32	CHRYSENE	78.180	82.107	87.780	81.120	1.650	5.230	2.670	0.34

CONCLUSION

It was demonstrated that an enhanced analytical technique currently developed and validated was specific, sensitive, accurate, and precise, with broad linear ranges for the analysis of semi-volatile organic compounds in parenteral formulation sample i.e Sumatriptan Injection USP (6 mg/0.5 mL). The above method can identify and quantify the unknown potentially leached semi-volatile organic compounds from the parenteral formulation. Hence, this method can be effectively applied for regular analysis.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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