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Simultaneous Separation and Detection of Two Genotoxic Impurities in Urapidil Hydrochloride by HS-GC

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ABSTRACT

The method focuses on using headspace gas chromatography (HS-GC) to separate and detect two possible genotoxic impurities (benzene and chloroethane) in urapidil hydrochloride API. The analysis was conducted using an Agilent DB-624 GC column to separate two possible genotoxic impurities. A FID detector fitted to the Shimadzu 2010 Pro GC was used to quantify two possible genotoxic impurities in sample. Initially, the column is set to 40°C and maintained at this temperature for 1 min to ensure sample equilibration. Subsequently, the temperature is raised to 80°C at a rate of 20°C per minute, followed by a 10-min hold phase to achieve optimal separation resolution. After completing this gradient segment, the temperature is further elevated to 220°C at an increased rate of 50°C per minute, with a final 5-min hold to complete the elution process. A detector and an injection port, both set to 220°C, are utilized. Furthermore, the headspace vial is heated to 85°C and held at this temperature for 20 min to achieve equilibration. This experiment uses nitrogen as a carrier gas, flowing at 0.5 mL per minute with a split ratio of 20:1. For two genotoxic impurities, the suggested approach yields strong linear relationships between LOQ to 200% limit level, with R^2 is above the threshold of 0.990. The accuracy results, which range from 96.17% to 101.33% for benzene and 94.28% to 99.92% for chloroethane, further demonstrated the method's effectiveness. Additionally, both the repeatability and intermediate precision RSD values fell below the acceptable criteria. The method demonstrated satisfactory results in accordance with International Council of Harmonization (ICH) requirements and can be utilized for urapidil hydrochloride daily quality control.

Keywords: Genotoxic impurity, Urapidil hydrochloride, ICH M7, HS-GC.

INTRODUCTION

Urapidil Hydrochloride, is a type of uracil compound as shown in Fig. 1 [1,2,3], it was developed by German firm BYKGULDEN[4] and is widely utilized for managing essential hypertension, as well as administered intravenously in cases of hypertensive emergencies[5,6], pheochromocytoma,[7] renal ischemia reperfusion-related renal injury[8]. Until now the research of urapidil were focused on stress degradation products[2], synthetic and hydrolytic impurities[9,10], metabolites[11], assay[12] and residual solvent[13] etc, but there are no reports of genotoxic impurities research.

Benzene is a solvent that may be carried

in ethanol[14], dichloromethane, and hydrochloric acid[15]. Hydrochloric acid may react with ethanol to form chloroethane[16]. Due to the usage of ethanol, dichloromethane, and hydrochloric acid in the production process, urapidil hydrochloride may produce genotoxic impurities such as benzene and chloroethane. A number of studies indicate that neither benzene nor its metabolites are effective mutagens, but they are highly clastogenic, causing sister chromatid exchanges, and the formation of micronuclei in animals, well as cause cancer in humans in various forms[17,18]. Humans are strongly suspected of developing cancer from chloroethane because it is a known animal carcinogen. When high amounts of chloroethane and its metabolites are circulating in most organs, Phase II conjugation metabolic elimination reduces tissue essential glutathione pools, which are then reduced by glutathione transferase[19,20,21]. Consequently, the presence of benzene and chloroethane in urapidil hydrochloride should be investigated and controlled.

As outlined in the ICH guideline "Q3C" 2024, benzene is classified as a Category 1 solvent, which has a defined concentration limit (2 ppm)[22]. The IARC has classified chloroethane as a Class 3 carcinogen, indicating its potential to pose a carcinogenic risk to humans, and ICH M7(R2) also provided the acceptable intakes based on TD50 linear extrapolation method and requiring a limit of no more than 1810 µg/day. There are several studies that use GC[17] or GC/MS[21] to identify benzene and chloroethane in medicines. In particular, GC/MS are not widely accessible in pharmaceutical manufacturing facilities and need highly skilled personnel. The authors employed the DB-624 column in conjunction with headspace sampling(HS) and a flame ionization detector (FID) for this investigation.

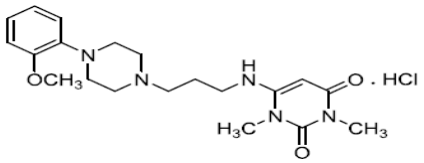

Fig. 1. Chemical structure of urapidil hydrochloride

MATERIAL AND METHODS

A FID detector fitted to the Shimadzu 2010 Pro GC was used to quantify two possible genotoxic impurities in sample. A Sartorius SQP and CPA225D electronic balance were used for weighing references and test samples. The gas chromatography is equipped with a fused-silica capillary column coated with 6% cyanopropylphenyl-94% dimethylpolysiloxane (DB-624, 30m×0.32mm, 1.8µm). HPLC grade distilled water was prepared by Arium® Pro Ultrapure Water Purification System (Sartorius Corporate Administration GmbH).

Materials and Chemical

The investigated sample (Batch No. 221001~221003) were provided by Chengdu Aupone Pharmaceutical Co., Ltd. Chloroethane in Methanol (5000 µg/mL, Batch No.22040943) were sourced from Tan-Mo Technology Co., Ltd. Benzene (Batch No. 2019102301), N,N-dimethylformamide (Batch No. 2019121128), Methanol (Batch No. 2021042926), Ethanol (Batch No. 2022092101), Acetonitrile (Batch No. 2021052519) and Dichloromethane (Batch No. 2022101401) were obtained through Chengdu Chron Chemical Technology Co., Ltd.

Conditions of chromatographic method

The column temperature profile is designed as follows: Initially, the column is set to 40°C and maintained at this temperature for 1 min to ensure sample equilibration. Subsequently, the temperature is raised to 80°C at a rate of 20°C per min followed by a 10-min hold phase to achieve optimal separation resolution. After completing this gradient segment, the temperature is further elevated to 220°C at an increased rate of 50°C per min with a final 5-min hold to complete the elution process. A detector and an injection port, both set to 220°C. The headspace vial is also maintained at 85°C for 20 min to achieve equilibration. This experiment uses nitrogen as a carrier gas, flowing at 0.5 mL per minute with a split ratio of 20:1. The test solution and reference solution should be injected separately onto the column, and the chromatogram should be recorded separately. Accurately dispense 5 mL of each solution into a 20 mL headspace vial and seal it securely.

Preparation of solutions

Preparation of diluent

A 1:1 solution of DMF and water was used as diluent.

Benzene stock solution

A 50 mg portion of benzene was accurately quantified and introduced into a 50 mL volumetric flask. Dimethylformamide (DMF) was added incrementally until the solution reached the calibration line, and the mixture was homogenized via vigorous shaking. Subsequently, 1 mL of this solution was aspirated and transferred to a 100 mL volumetric flask. The flask was brought to volume with diluent, and the diluted solution underwent 30 sec. of vortex agitation to ensure complete homogeneity.

Benzene positioning solution

In this phase of the experimental procedure, a precisely measured 1 mL aliquot from the benzene stock solution was carefully transferred into a 25 mL calibrated volumetric flask. Following this transfer, the dilution process commenced by gradually introducing the diluent solution through the flask's neck. The liquid level was meticulously adjusted until the meniscus aligned perfectly with the flask's calibration mark at room temperature, and the mixture was vortex-mixed for 30 sec. to ensure homogeneity.

Chloroethane stock solution

A 1 mL of chloroethane (diluted in methanol solution, 5000 µg/mL) was accurately quantified and introduced into a 20 mL volumetric flask The diluent was added until the solution reached the mark, and the mixture was vortex-mixed for 30 sec. to ensure homogeneity.

Chloroethane positioning solution

A 1 mL aliquot of the chloroethane stock solution was accurately pipetted into a 25 mL volumetric flask. The diluent was added until the solution reached the mark, and the mixture was vortex-mixed for 30 sec. to ensure homogeneity.

Standard solution

1 mL of the benzene stock solution and 2 mL of the chloroethane stock solution were accurately dispensed using a calibrated pipette, followed by transfer into a 50 mL volumetric flask. The solution was then brought to volume by adding the diluent until the calibration mark was reached, ensuring precise volumetric determination, and the mixture was vortex-mixed for 30 sec. to achieve a uniform and homogeneous solution.

Sample solution

The sample (approximately 500 mg) was carefully measured and introduced into a 20 mL headspace vial. Following this, the diluent was dispensed in an exact volume of 5 mL into the container, which was subsequently hermetically sealed and the mixture was vortex-mixed for 30 sec. to ensure homogeneity.

Mix stock solution

Based on pre-calculated stoichiometric ratios derived from the formulation protocol, methanol, ethanol, acetonitrile, and dichloromethane were individually metered into designated 100 mL volumetric flasks according to their respective mass percentages. Each solvent was quantitatively delivered, after which the flasks were brought to volume with diluent and the mixture was vortex-mixed for 30 sec. to ensure homogeneity.

Mix positioning solution

A homogenized three-component solution was prepared by pipetting 1 mL of the stock solution, benzene calibration standard, and chloroethane reference material into a 50 mL volumetric flask. The solution was then brought to volume by adding the diluent until the calibration mark was reached, ensuring precise volumetric determination, and the mixture was vortex-mixed for 30 sec. to ensure homogeneity.

Mix sample solution

Precisely 500 mg (±0.5 mg) of test material was portioned into a sterile 20 mL headspace vial using analytical-grade spatulas. A 5 mL volume of diluent was dispensed via calibrated pipette into the containment vessel, which was immediately crimp-sealed and the mixture was vortex-mixed for 30 sec. to ensure homogeneity.

Spiked standard solution 100% Spiked Standard Solution

The standard solution itself was used as the 100% spiked standard solution. 50% Spiked Standard Solution: To obtain the target mixture, a calibrated pipette was used to deliver 0.5 mL of benzene stock solution into a 50 mL volumetric flask. This was followed by the addition of 1 mL chloroethane stock solution, ensuring both components were proportioned as

required. The diluent was added until the solution reached the mark, and the mixture was vortex-mixed for 30 seconds to ensure homogeneity.

150% Spiked Standard Solution: To obtain the target mixture, a calibrated pipette was used to deliver 1.5 mL of benzene stock solution into a 50 mL volumetric flask. This was followed by the addition of 3 mL chloroethane stock solution, ensuring both components were proportioned as required. The diluent was added until the solution reached the mark, and the mixture was vortex-mixed for 30 sec. to ensure homogeneity.

Spiked sample solution

Each 20 mL headspace vial received a precisely measured aliquot of approximately 500 mg sample, which had been carefully portioned into individual containers prior to subsequent processing. Different concentrations (50%, 100%, and 150%) of the spiked standard solution were added precisely at 5 mL each to the respective vials. The vials were sealed and shaken thoroughly to ensure proper mixing. Each concentration was prepared in triplicate to ensure reproducibility.

RESULTS AND DISCUSSIONS

Optimization of Chromatographic Conditions

The most important criterion to take into account was the resolution (any two peaks' resolution should be more than 1.5)[20,21]. The peak height (sensitivity), symmetry factor (between 0.8 and 1.5), theoretical plates (more than 5000), and method run time (less than 60 min) should be considered.[23,24]

Based on the residual solvent method of urapidil in the Chinese Pharmacopoeia II, a preliminary analysis method for benzene and chloroethane was established. It is important to consider whether methanol and ethanol affect the detection of benzene and chloroethane, as ethanol was found in the urapidil hydrochloride sample and chloroethane reference substance was a methanol solution.

Appropriate separation conditions were finally chosen by screening the chromatographic column, optimizing the programmed heating program, and raising the split ratio.

Different polar chromatographic columns were chosen for screening because three residual solvents (ethanol, acetonitrile, and dichloromethane), one reference substance diluent (methanol), and two genotoxic impurities (chloroethane and benzene) must be separated. HP-5, DB-FFAP, DB-624, and DB-WAX were the columns that were analyzed; according to the impurities discovered, each had a different polarity, with methanol being extremely polar and benzene being less polar. Separation would not be possible with a column that is either too polar or too low. The widely used DB-624 chromatographic column for residual solvents was finally selected following experimental comparison, and all six compounds were fully separated on it. The final chromatographic conditions meet the set principles: the resolution of each impurity is greater than 1.5, almost all peaks have a greater S/N than 50, the peak shape is symmetrical, the theoretical plate number is greater than 20000, and the analysis time is less than 30 minutes.

Optimization of diluent

Dimethyl sulfoxide (DMSO), DMF, water, and various DMF-water solution ratios were investigated. Although the sample could completely dissolve in DMSO, DMSO tends to degrade into methyl thioether, dimethyl disulfide, and methyl sulfide under acidic conditions due to the sample being a hydrochloride salt[25,26], which may cause interference. DMF was also considered as a solvent, but it showed very low response for benzene, almost no peaks appeared. Pure water as a solvent could easily damage the gas chromatography column, so various DMF-water solutions were tried instead[27,28]. Higher sensitivity for most solvents, especially Class 1 solvents was obtained with DMF-water solutions.Benzene and chloroethane reaction comparisons for various DMF-water solution ratios are shown in Fig. 1. The ultimate choice of 10% DMF as the diluent was made because the findings show that while the response of benzene as the ratio of DMF increases, there is almost no difference in the response of chloroethane to various ratios of DMF and water. Results indicate that chloroethane reacts roughly the same way in different combinations of DMF and water, while the response of benzene decreases with an increase in the ratio of DMF, leading to the final selection of 10% DMF as the diluent.

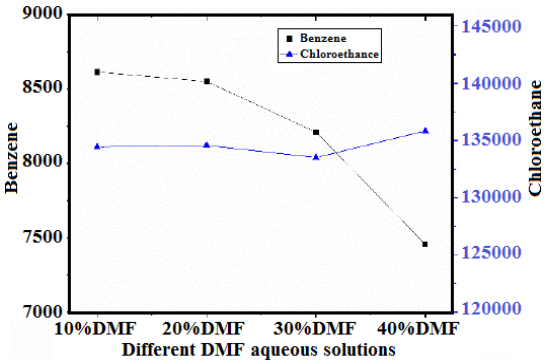

Fig. 1. The response of benzene and chloroethane in different DMF aqueous solutions

Optimization of chromatographic method

An analytical method for benzene and chloroethane in urapidil hydrochloride was established according to the Chinese Pharmacopoeia (Part II). A polyethylene glycol (PEG)-based capillary column (DB-WAX, 30 m×0.32 mm, 0.5 μm) was employed. The initial oven temperature was set at 40°C for 2 min, followed by a temperature ramp at 5°C/min to 80°C and held for 10 min then rapidly increased at 50°C/min to 220°C and maintained for 5 minute. Preliminary results revealed partial co-elution of ethanol and benzene peaks, with a resolution of only 0.23, indicating potential interference from ethanol in the detection of benzene, see Fig. 2 for details. To address this issue, the temperature program was systematically optimized to achieve baseline separation of all target solvents and genotoxic impurities (chloroethane and benzene). Post-optimization chromatographic conditions successfully resolved critical peak overlaps, ensuring accurate identification and quantification of benzene in the presence of ethanol.

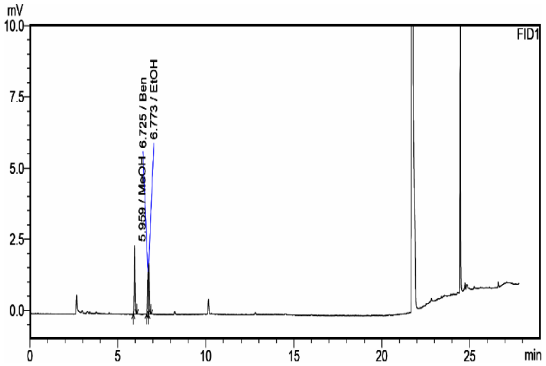

Fig. 2. The chromatogram obtained under unoptimized analytical conditions

Optimization of headspace equilibrium temperature

To avoid gas condensation during transmission, which might result in residue, the heating furnace's temperature is gradually raised from the furnace temperature, transfer line temperature, and quantitative loop temperature to the transfer line temperature[29]. Consequently, the ideal range for the furnace temperature is one that is greater than the target substance's boiling point but lower than the diluent's.The boiling point of benzene is 80.1°C, chloromethane is -24.2°C, water is 100.0°C, and DMF is 153°C. To evaluate system performance under thermal variation, testing was performed across a controlled temperature gradient spanning 75°C to 90°C, with experimental parameters systematically adjusted in 5°C increments, as detailed in Fig. 3. Higher temperatures often resulted in faster dynamic equilibration and greater extraction yields; The procedure reached its optimal condition when mass transfer to the headspace (HS) was nearly instantaneous and complete[30]. When the temperature exceeds 100°C, decomposition peaks will appear in the sample, affecting its determination; when the temperature is below 80°C, the response of benzene is low. In order to guarantee full volatilization of benzene and chloromethane in the sample while reducing the volatilization of background water, the equilibrium temperature is finally fixed at 80°C after screening.

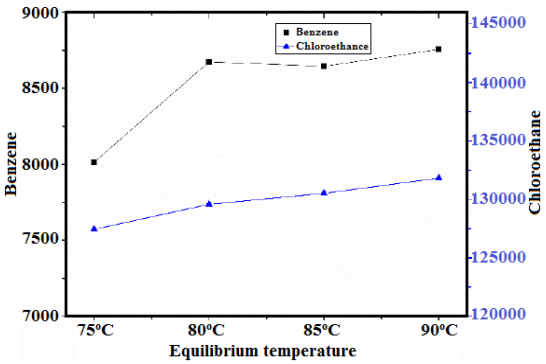

Fig. 3. The result of optimization of headspace equilibrium temperature

Optimization of headspace equilibrium time

Another important consideration for HS was equilibration time, which was directly related to the establishment of equilibration between the two phases[31]. The gas and liquid phases are unable to attain equilibrium when the equilibrium time is short. The headspace vial's airtightness deteriorates

once the equilibrium period surpasses 60 min which lowers the chromatogram's peak area[29]. A comparison was made between the 15-min, 20-min, 25-min and 30-min responses, as detailed in Fig. 4. Considering both peak response and cost, an equilibrium time of 20 min was ultimately selected to meet the detection requirements.

Analytical methodology and validation

System suitability

To establish method precision, a six-injection replicate analysis of the reference solution was conducted under isocratic chromatographic conditions. System suitability parameters were quantified through statistical determination of relative standard deviation (RSD%) for both retention time precision (t_R) and integrated peak area reproducibility across the benzene-chloroethane analyte pair, together with theoretical plate numbers for both compounds. The theoretical plate numbers for chloroethane were all greater than 50,000, and the RSD values of retention times for both chloroethane and benzenewere 0.01%. The RSD values of peak areas were 2.63% for chloroethane and 2.27% for benzene. In Table 1, the theoretical plate counts and %RSD in compliance with ICH guidelines.

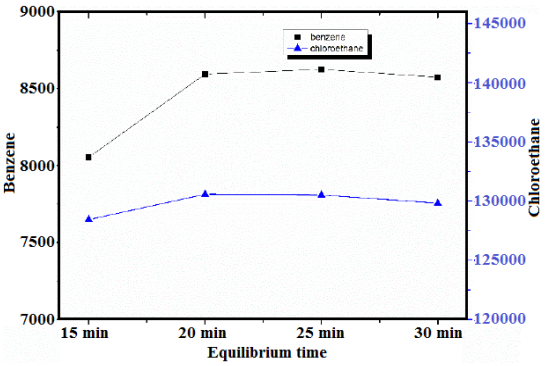

Fig. 4. The result of optimization of headspace equilibrium time

Table 1: System suitability of the optimization method

No	Chloroethane				Benzene.				
	TP*	RT#	PA&	TP@	TP*	RT#	PA&	TP@	RS*
1	56644	7.451	138252	1.01	346644	14.074	8268	1.04	60.01
2	58394	7.451	131945	1.01	348394	14.073	7888	1.04	61.37
3	58605	7.450	139611	1.02	348605	14.073	8297	1.04	60.90
4	57722	7.452	136435	1.01	347722	14.075	8004	1.00	60.65
5	58285	7.450	130528	1.01	348285	14.073	7895	1.02	60.80
6	58294	7.451	136542	1.02	348294	14.072	7973	1.04	60.52
	mean	7.451	135552	/	/	14.073	8054	/	/
	RSD(%)	0.01	2.63	/	/	0.01	2.27	/	/

TP*: Theoretical plate; RT#: Retention time(min); PA &: Peak Area; TF@: Tailing Factor; RS: Resolution

Specificity

Several chromatograms were recorded with separate injections of Blank solution, benzene positioning solution, chloroethane positioning solution, standard solution, sample solution, and mix sample solution. The results are detailed in Fig. 5 to 10. The results show that the blank, test sample, methanol, ethanol, acetonitrile, and dichloromethane do not interfere with the detection of benzene and chloroethane.

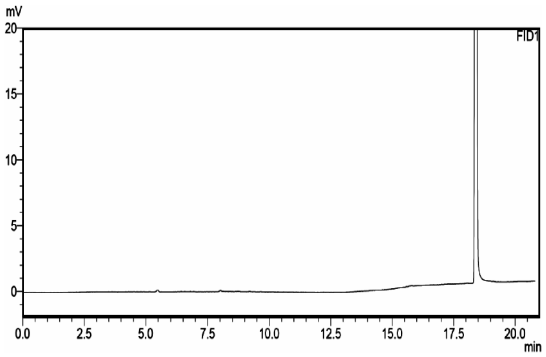

Fig. 5. The HS-GC chromatogram of blank solution

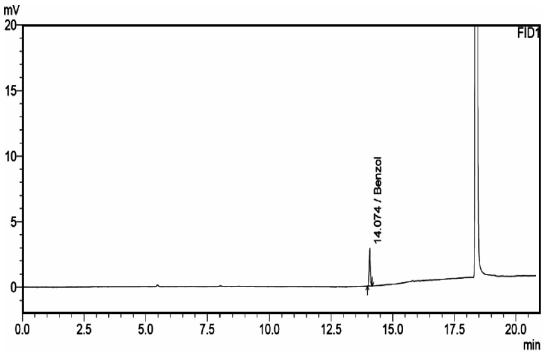

Fig. 6. The HS-GC chromatogram of benzene positioning solution

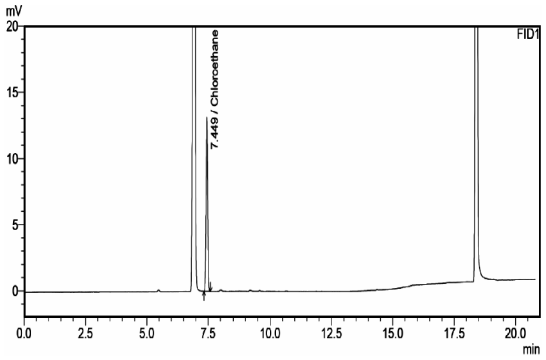

Fig. 7. The HS-GC chromatogram of chloroethane positioning solution

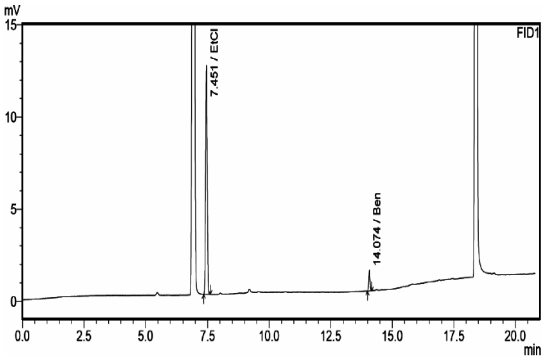

Fig. 8. The HS-GC chromatogram of standard solution

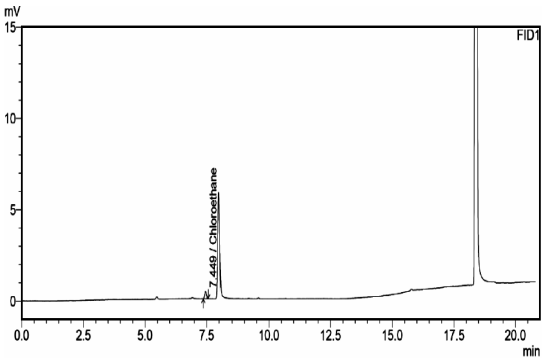

Fig. 9. The HS-GC chromatogram of sample solution

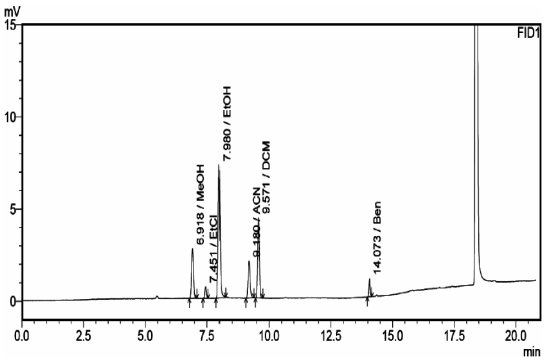

Fig. 10. The HS-GC chromatogram of mix sample solution

(MeOH: methanol; EtCl: choroethane; EeOH: ethanol; ACN: acetonitrile; DCM: dichloromethane; Ben: benzene)

Limits of Detection and Limits of Quantification

In accordance with ICH Q2(R1) validation guidelines, the limit of detectability(LOD) and quantitation (LOQ) were established to assess the method's sensitivity[32]. The LOQ and LOD levels were predicted by the estimation of the S/N NLT10 and NLT3, respectively. The experimental findings have been systematically organized in tabular format (Table 2) for comparative analysis. Six injections of a solution containing two genotoxic impurities from a level of 0.027 µg/mL (benzene, 0.000027%) to 0.100 µg/mL (chloroethane, 0.0001%) were used to show the LOQ; and three injections of a solution containing two genotoxic impurities from a level of 0.013 µg/mL (benzene, 0.000014%) to 0.050 µg/mL (chloroethane, 0.00005%) were used to LOD.

Table 2: The LOQ and LOQ for the optimized method

		Chloroethane				Benzene			
	No	S/N	Peak Area	Mean	RSD(%)	S/N	Peak Area	Mean	RSD(%)
	1	16.60	1017			12.21	1011		
LOQ	2	20.51	1047			15.26	1044		
	3	15.01	1030	1033	2.16	11.15	1104	1039	5.93
	4	18.55	1070			14.13	1118		
	5	15.90	1011			11.74	962		
	6	16.48	1020			12.22	997		
LOD	1	4.53	372	385	5.99	6.61	419		
	2	3.87	372			5.79	457	444	4.82
	3	4.42	412			6.36	455		

Linearity and range

In order to validate linearity, the stock solution of benzene and chloroethane was quantitatively diluted to 200%, 150%, 100%, 50%, 20% and LOQ. For the two impurities, a correlation coefficient greater than 0.990 was observed,

demonstrating that the proposed GC method exhibits linearity within the tested concentration range. The measured peak area are plotted versus concentration to create calibration curves. Fig. 11 to 12 show the calibration curves for both impurities, and Tables 3 to 4 provide the linearity parameters associated with them.

Table 3: The linearity and range for chloroethane (n=3)

Limit level	Concentration (µg/mL)	Peak area
LOQ	0.10	1011
20%	2.00	27895
50%	5.00	65026
100%	10.00	135402
150%	15.00	207202
200%	20.00	271662
Linearity Range (µg/mL)		0.10~20.00
Regression Equation		y=13682.6186x-777.7384
Correlaiton codfficient(R^2)		0.9997

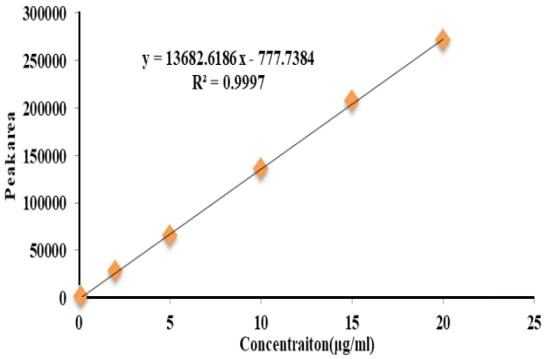

Fig. 11. Calibration curves for chloroethane

Table 4: The linearity and range for benzene

Limit level	Concentration (µg/mL)	Peak area
LOQ	0.027	1037
20%	0.043	1570
50%	0.108	4206
100%	0.215	8447
150%	0.323	12509
200%	0.430	17562
Linearity Range(µg/mL)		0.027~0.43
Regression Equation		y=40514.5478x-183.1120
Correlaiton codfficient(R^2)		0.9987

Repeatability

By injecting six duplicates of the sample solution with preset acceptance criteria for a percent RSD of <5.0% for repeatability. As shown in Table 5, the %RSD of outcomes was within the limits.

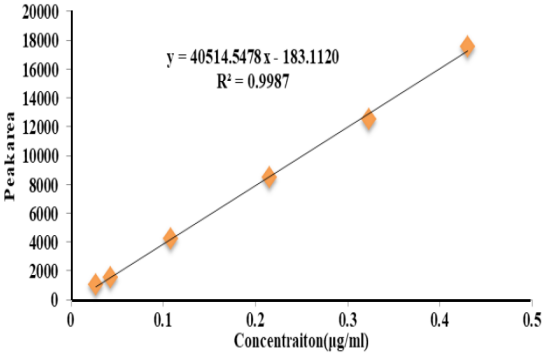

Fig. 12. Calibration curves for benzene

Table 5: Repeatability results

Repeatability	Weight(mg)	Choroethane		Benzene	
		Area	Impurity(%)	Area	Impurity(%)
1	501.25	1874	0.00021	ND	ND
2	505.38	1987	0.00022	ND	ND
3	493.25	1925	0.00023	ND	ND
4	523.10	1896	0.00020	ND	ND
5	527.52	1952	0.00021	ND	ND
6	489.31	1889	0.00022	ND	ND
	Mean (n=6)		0.00021	NA	NA
	RSD (n=6,%)		4.79	NA	NA

ND: Not Detected; NA: Not Applicable; the same below.

Intermediate precision

By injecting six duplicates of the sample solution by different person, instrument, and date with preset acceptance criteria for a percent RSD of <10% for intermediate precision. As can be seen from Table 6 of the results, the %RSD of the results was within the acceptable boundaries of what was considered acceptable.

Table 6: Intermediate precision results

Intermediate precision	Weight (mg)	Choroethane		Benzene	
		Area	Impurity(%)	Area	Impurity(%)
1	503.77	1987	0.00021	ND	ND
2	512.01	1921	0.00021	ND	ND
3	499.38	1889	0.00021	ND	ND
4	520.25	1875	0.00019	ND	ND
5	512.56	1898	0.00020	ND	ND
6	497.52	1991	0.00022	ND	ND
	mean (n=6)		0.00021	NA	NA
	RSD (n=6,%)		4.16	NA	NA
	mean (n=12)		0.00021	NA	NA
	RSD (n=12,%)		4.67	NA	NA

Accuracy

Method accuracy was verified through spiked recovery studies for two genotoxic impurities at 50%, 100%, and 150% spiked levels. As shown in Table 7, recoveries ranged from 90%

to 110%, meeting the validation criteria of ICH Q2(R1).

$$\text{Accuracy} = \frac{\text{Amount recovered} - \text{Original amount}}{\text{Amount added}} \times 100\%$$

Table 7: Accuracy results

Level	Weight (mg)	Original Amount (µg)	Amount Added (µg)	Peak area	Amount Recovered (µg)	Accuracy (%)	Mean (%)	RSD (%)
Benzene-50%	509.15	0	0.5375	3906	0.4971	92.48	96.17	4.42
	504.58			4021	0.5117	95.21		
	495.20			4258	0.5419	100.82		
Benzene-100%	489.63	0	1.0750	8259	1.0511	97.77	101.33	3.31
	505.69			8598	1.0942	101.79		
	512.58			8821	1.1226	104.43		
Benzene-150%	491.26	0	1.6125	12987	1.6528	102.50	99.98	4.52
	509.76			13010	1.6557	102.68		
	512.63			12006	1.5279	94.76		
Chloroethane-50%	509.15	1.0692		66894	24.1075	92.15	94.28	3.01
	504.58	1.0596	25.0000	67582	24.3555	93.18		
	495.20	1.0399		70521	25.4146	97.50		
Chloroethane-100%	489.63	1.0282	50.0000	134579	48.5001	94.94	98.49	3.87
	505.69	1.0619		138921	50.0649	98.01		
	512.58	1.0764		145219	52.3346	102.52		
Chloroethane-150%	491.26	1.0316	75.0000	217158	78.2602	102.97	99.92	2.78
	509.76	1.0705		209517	75.5065	99.25		
	512.63	1.0765		205981	74.2322	97.54		

Solution stability

The stability of sample and standard solutions was evaluated by storing both solutions at ambient temperature (25 ± 2°C) over a 24-h period. Aliquots were analyzed at predetermined intervals (0, 3, 9, 12, 16, and 24 h) under the validated method conditions. Solution stability was considered acceptable if the relative standard deviation (RSD) across all time points remained below 10%, aligning with the predefined analytical acceptance criteria of the International Council for Harmonisation (ICH) Q2(R1) guidelines. In order to determine the peak area of each impurity, the chromatograms of each impurity were recorded and the peak area was determined over time. Table 8 summarizes the complete results.

Table 8: The standard solution and sample solution stability

Time	Standard Solution		Sample Solution	
	Benzene	Choroethane	Benzene	Choroethane
0 h	8448	130725	ND	3021
3 h	8623	128320	ND	2751
9 h	8395	135906	ND	2958
12 h	8040	130586	ND	3079
16 h	8319	132320	ND	2702
24 h	8291	128544	ND	2958
Mean	8353	131067	NA	2912
RSD (%)	2.31	2.14	NA	5.19

Robustness

The method's robustness was systematically verified in accordance with ICH Q2(R1) regulatory framework by implementing controlled variations of core analytical variables. Key system parameters underwent adjustments encompassing: (1) initial column temperature (±5°C), (2) carrier gas delivery rate (±0.1 mL/min), (3) injection port temperature (±5°C), and (4) FID thermal settings (±5°C). Additional assessments involved one distinct chromatographic condition (different chromatographic column, instrument, and analysts). All experimental deviations met the predefined acceptance criteria (RSD<10.0%), with comprehensive data presented in Table 9. This protocol aligns with the ICH Q2(R1) recommendations for robustness testing in analytical method validation.

Table 9: Robustness results

Parameters	RSD%	
	Benzene	Choroethane
Initial column temperature 35°C	ND	0.00021
Initial column temperature 45°C	ND	0.00023
flow rate 0.4 mL/min	ND	0.00020
flow rate 0.6 mL/min	ND	0.00024
injection port temperature 195°C	ND	0.00022
injection port temperature 205°C	ND	0.00021
FID temperature 215°C	ND	0.00023
FID temperature 225°C	ND	0.00021
Column II & Instrument II & Analyst II	ND	0.00021
Mean	NA	0.00022
RSD%	NA	5.98

Real sample detection

The suggested method was used to analyze many lots of urapidil hydrochloride (221001~221003). Peak areas were used to assess each chromatogram, and the outcomes are shown in Table 10. The chromatograms revealed that all samples no included benzene, and the detected amount of chloroethane is far below the 5% control threshold. It was thus shown that the suggested method might be used to the regular quality control of genotoxic impurities in urapidil hydrochloride.

Table 10: Real sample detection results

			Beneze			Chloromethan	
Batch No.	Weigh (mg)	Control threshold (ppm)	Peak area	Assay(%)	Threshold(%)	Peak area	Assay(%)
221001	500.36	2	ND	NA	0.01	1142	0.00020
221002	506.47	2	ND	NA	0.01	1862	0.00032
221003	499.84	2	ND	NA	0.01	1979	0.00034

CONCLUSION

The establishment and validation of a headspace gas chromatography (HS-GC) methodology facilitated concurrent resolution and quantitative monitoring of two genotoxic impurities-benzene and chloroethane-in urapidil hydrochloride drug substance. Method validation studies revealed exceptional linear correlation (R^2>0 990) across the validated concentration range (LOQ-200%), and precision (RSD<5.0%) conforming to stringent validation thresholds prescribed in ICH Q2(R1) for chromatographic impurity methods. The accuracy results, which range from 96.17% to 101.33% for benzene and 94.28% to 99.92% for chloroethane. Method validation, including specificity, sensitivity, and robustness, also complied with ICH Q2(R2) recommendations for genotoxic impurity control. This cost-effective analytical approach has been successfully implemented for routine quality monitoring of urapidil hydrochloride in commercial batches.

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Conflict of interest

The author declare that we have no conflict of interest.

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