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Development of Gas Chromatographic method for Carcinogens

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Abstract

A simple, fast and accurate method has been developed for the determination of Benzene in Toluene by Gas Chromatography. The analysis was carried out on Shimadzu GC-2010 Gas Chromatograph. The column used was 30mx 0.32 mm Id fused silica analytical column ZB-624, 1.80 μm. (6% cyanopropylphenyl: 94% dimethylpolysiloxane as a stationary phase). The detector used was FID detector. The validation of proposed method was also carried out.

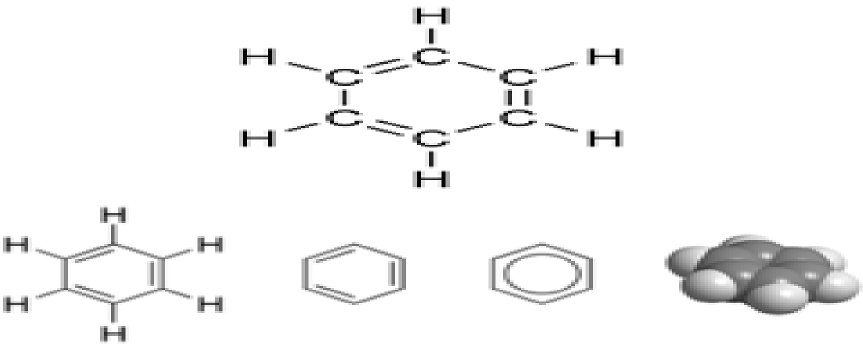
Key words : Gas Chromatography, Benzene, Toluene.

Introduction

From mobile phones to DVDs, from car headlights to high-performance skis, many everyday products are made from materials that can trace their beginnings back to benzene. Benzene is a very important basic chemical, being converted into many of the intermediates and polymers which are needed to produce a wide range of the goods we all use everyday. Familiar products that may be made from chemicals derived from benzene include clothing, packaging, paints, adhesives, unbreakable windows, plywood, computer casings, compact discs and many more.Benzene has always been present in small quantities in gasoline but its presence is currently being minimised, in-line with changing legislation to reduce exposure to benzene.

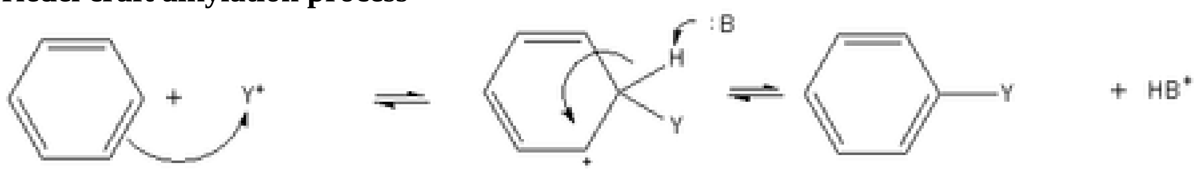
Benzene is an organic chemical compound and a known carcinogen with the molecular formula C_6H_6. It is sometimes abbreviated Ph–H. Benzene is a colorless and highly flammable liquid with a sweet smell and a relatively high melting point. Because of this, its use as an additive in gasoline is now limited, but it is an important industrial solvent and precursor in the production of drugs, plastics, synthetic rubber, and dyes. Benzene is a natural constituent of crude oil, but it is usually synthesized from other compounds present in petroleum. Benzene is an aromatic hydrocarbon and the second [*n*]-annulene ([6]-annulene), a cyclic hydrocarbon with a continuous pi bond.

Many important chemicals are derived from benzene, wherein with one or more of the hydrogen atoms is replaced with another functional group. Examples of simple benzene derivatives are phenol, toluene, and aniline, abbreviated PhOH, PhMe, and $PhNH_2$, respectively. Linking benzene rings gives biphenyl, C_6H_5–C_6H_5. Further loss of hydrogen gives "fused" aromatic hydrocarbons, such as naphthalene and anthracene. The limit of the fusion process is the hydrogen-free material graphite.

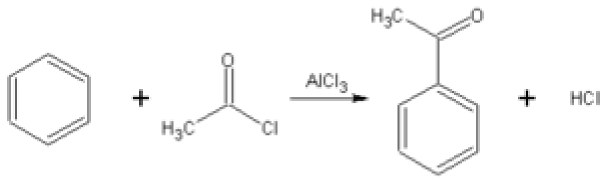


Benzene structure

Friedel craft alkylation process



Electrophilic aromatic substitution



Friedel craft acylation process

In Pharmaceutical industry, manufacturing of Chemicals or drugs require use of many solvents. Residue of this solvent in pharmaceuticals are known as Organic volatile impurities. These solvents are not completely removed by practical manufacturing techniques. Appropriate selection of organic solvent in a synthesis of drugs enhance yield of drugs, or determine characteristic of such critical parameter as crystal form, purity and solubility. It means it plays an important role in the synthetic process. Since there are no therapeutic benefits from residual solvents, all solvents should be removed to the extent possible to meet product specification, Good manufacturing practices or other quality based requirements. Depending upon toxicity residual solvents are classified into four classes internationally. Limits for residual solvent are fixed based on TDI (Tolerable daily intake) or PDE (Permitted daily exposure). Nowadays Benzene is totally banned while manufacturing of pharmaceuticals. But industries using Toluene in manufacturing of active pharmaceuticals or intermediates may contain some amount of benzene into it. As per ICH Guidelines Limit of Toluene is 890 ppm. So it is highly recommended to check or monitor traces of benzene level in Toluene. Since Toluene is used in final stage, it is necessary to check benzene in toluene and control the same.

Cancer is caused by abnormalities in a cell's DNA. These may be inherited from parents, or they may be caused by outside exposures to the body such as chemicals, radiation, or even infectious agents. Study shows that Benzene is Carcinogenic compound. The developed method is used to determine Benzene levels in Toluene quantitatively.

Carcinogens do not cause cancer in every case, all the time. Substances classified as carcinogens may have different levels of cancer-causing potential. Some may cause cancer only after prolonged, high levels of exposure,and for any particular person, the risk of developing cancer depends on many factors, including the length and intensity of exposure to the carcinogen and the person's genetic makeup.

Manufacturing process

Benzene and Toluene are manufactured from stabilized reformat produced in the reformer. The feed to reformer unit is 60-90°C cut Naphtha which is produced in a feed preparation unit. Benzene and Toluene are extracted from the reformat with the help of Sulfolane Solvent in an extraction section. The extracted aromatics are separated by fractionation to produce Benzene and Toluene separately. The Sulfolane solvent is recovered and reused in the extraction plant. Benzene and Toluene are pure aromatic hydrocarbon compounds having fixed boiling points, unlike other petroleum products which are mixtures of hydrocarbon compounds. They are colorless and flammable liquids possessing characteristic aromatic odour. The boiling points of Benzene and Toluene are 80.1°C and 110.6°C respectively. Benzene is slightly soluble in water whereas Toluene is virtually immiscible with water.

Material and methods

Reagents and chemicals: Benzene A.R.grade make :E Merck India Ltd.
Toluene HPLC grade : E Merck India Ltd.

Chromatographic Conditions: Column type : 30 m x 0.32 mm Id fused silica analytical column ZB-624, 1.80 µm.(6% cyanopropylphenyl 94 % dimethylpolysiloxane)

Carrier gas	:	Helium
Makeup gas	:	Helium
Carrier gas flow rate	:	5.0 ml/min.
Makeup gas flow rate	:	30.0 ml/min.
Detector	:	FID
Injector port temperature	:	220°C
Injection mode	:	Split
Detector temperature	:	250°C
Equilibration time	:	1 min.
Column oven temperature	:	Initially hold at 60°C and then increase at the rate of 10°C/min to 150ºC and hold for 5 min.
Split ratio	:	20:1

Instrument :AOC- 5000 liquid sampler Parameters :

Cycle	: GC - injection.
Syringe	: 10 µl.
Sample volume	: 1.0 µl
Air volume	: 0
Pre cln Slv_1	: 5
Pre cln Slv_2	: 5
Pre cln Spl	: 5
Fill volume	: 5.0 µl
Fill speed	: 1 µl / Sec.
Fill stroke	: 5
Pull up delay	: 300 ms.
Inject to	: GC – inj 1.
Inject speed	: 50 µl/Sec.
Pre inj delay	: 00 ms
Post inj delay	: 2.0 s
Post cln Slv_1	: 3
Post cln Slv_2	: 3
Pre cln Spl	: 5

Preparation of Standard solution : Weighed accurately about 1.0 g of Benzene GC standards into a 200 ml volumetric flask containing about 120 ml of Toluene . Mixed and diluted to volume with toluene & shake vigorously. Further diluted 10 ml of this solution to 100 ml with Toluene. (Prepared in Duplicate)

Test solution : As such toluene sample

Procedure: 1.0 µl of standard solution and sample solution were injected into the Gas Chromatograph with the help of AOC- 5000 liquid sampler and chromatograms were recorded .The retention time obtained for benzene is 2.3 minutes.

Order of Elution : Benzene, and Toluene

Calculation *:*

Content of Benzene (ppm) :

$$= \frac{(A - B) \times W_1 \times 10 \times 10^6}{(C - B) \times 200 \times 100 \times}$$

Where,

A	=	Peak area response of benzene in test solution.
B	=	Peak area response of benzene interference from blank.
C	=	Average peak area response of benzene in standard solution B.
W_1	=	Weight of Benzene taken for standard solution B in g.
W_2	=	Weight of sample taken in g.

Results and Discussion

Linearity Experiment

The plot peak area of Benzene standards Vs respective concentration of linearity levels are found linear in the range of 25 ppm to 700 ppm with correlation 0.9992.Also LOQ Level and Higher level(Linearity 140%) injected in six replicates and determine the relative standard deviation which shows that method is precise for LOQ Level and Higher level (Linearity 140%)

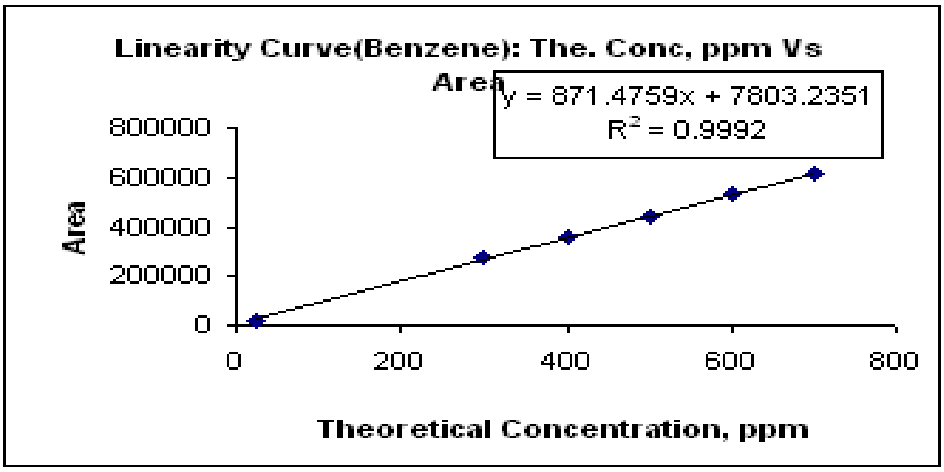
To determine the precision of the proposed method, Six samples (as such) were analyzed and determine the %RSD of Benzene content in Toluene. Method is also applicable for samples of different manufacturers of Toluene.

Recovery (Accuracy)

To study the accuracy, reproducibility and the precision of the proposed method, recovery experiment was carried out by adding standard Benzene at four different levels in pre-analyzed sample. The study was carried out with spiking of LOQ level (25 ppm),300 ppm ,500 ppm ,700 ppm.This was determined in triplicate. Recovery observed shown in following table.
Robustness study also performed on the sample by slightly changing the chromatographic parameters and found satisfactory.

LINEARITY

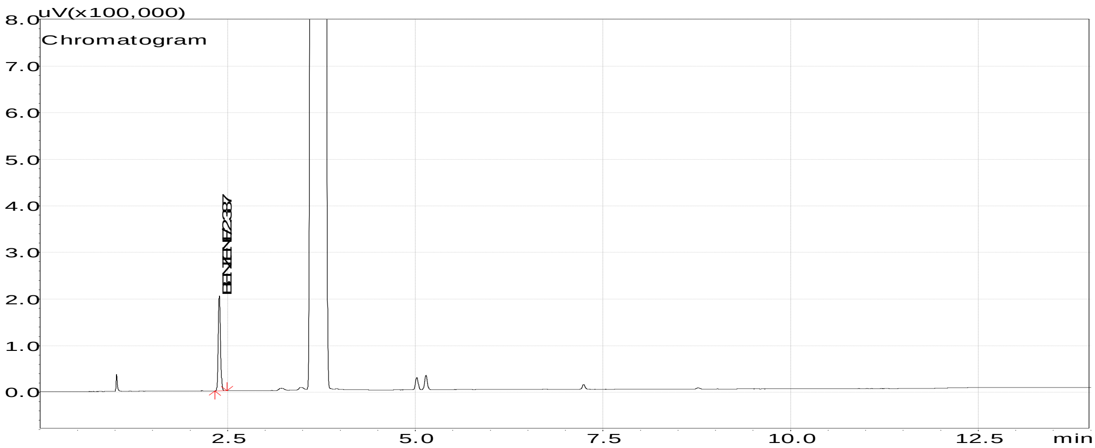
Sample Name	Conc. of Benzene(ppm)	Area of Benzene
LOQ Level	25	22723
Linearity 60%	300	277996
Linearity 80%	400	361504
Linearity 100%	500	442465
Linearity 120%	600	528863
Linearity 140%	700	613745



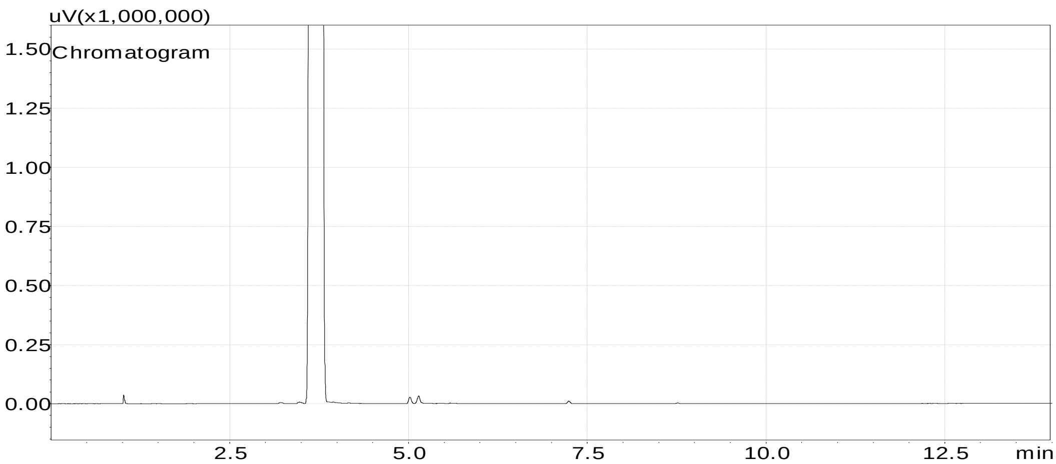
Accuracy (Recovery)

Sample Name	Sample Area	Amount Recovered	% Recovery	Average(%)	Std Dev	%RSD
LOQ Accuracy	23685	27.64	103.79665			
LOQ Accuracy	22798	26.60	101.14411	102.77	1.422	1.384
LOQ Accuracy	23076	26.93	103.35997			
60%Accuracy	277788	324.13	103.88635			
60%Accuracy	275532	321.49	103.70744	103.99	0.352	0.339
60%Accuracy	278680	325.17	104.38722			
100%Accuracy	441539	515.19	102.80185			
100%Accuracy	443998	518.06	103.35374	101.60	2.570	2.529
100%Accuracy	416406	485.87	98.653007			
140%Accuracy	608335	709.81	101.25684			
140%Accuracy	606989	708.24	101.09048	100.26	1.579	1.575
140%Accuracy	591423	690.08	98.441852			
Mean			101.95			
Std			2.10			
%RSD			2.06			

Chromatograms: Standard chromatogram of benzene (500 ppm) in toluene



Blank chromatogram of toluene



Conclusion

The linearity regression coefficient was found 0.9992 which shows that response is linear from 25 ppm to 700 ppm. High percentage of recovery shows that the method is free from interference of other raw material. The recovery value prove that method is accurate and reproducible .The proposed method is simple, fast, accurate and precise. Thus proposed method can be used For routine quality control analysis of Toluene for monitoring benzene content.

Acknowledgements

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