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RESEARCH ARTICLE

Comparison of residual styrene monomer determination of pharmaceutical materials packed with polystyrene using gas chromatography and ultraviolet/visible spectrophotometer

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ABSTRACT

Background: Plastics such as plastic wrapping are an integral part of today's society. Plastics are made by polymerization of styrene monomers. If the polymerization reaction is not perfect, then there will be unreacted monomer residues. Although styrene is still considered harmless, if there is a metabolic process will form styrene oxide that can cause effects that are carcinogenic. **Aims and Objectives:** This study aims to determine the residue of pharmaceutical styrene materials packed with polystyrene using gas chromatography (GC) and ultraviolet (UV)/visible spectrophotometers which are usually available in chemical analysis laboratories. **Materials and Methods:** Orientation of analysis method was performed using latex polystyrene sample. Styrene monomer analysis was performed using a GC and UV/visible spectrophotometer. Then a comparative study was conducted on both methods. Once this method was accepted, it was applied to a polystyrene-containing sample obtained from the market. Styrene was isolated from the sample; then, both methods were tested and compared. **Results:** Using polystyrene latex sample and varying column temperature, for GC, the optimum condition was obtained at column temperature 110°C, detector temperature 200°C, and temperature of injector 200°C. Using the packing column, we got a repeatability of 96.67%, PRECISION test of 2.81%, 96.49% recovery test, and a detection limit of 0.25 ppm. For the quantitative test of latex polystyrene samples, the average residual content obtained was 0.276%. When using GC with a capillary column, used column DB-17 at 110°C, detector, and injector temperature at 200°C, the average content of styrene residue was 0.278%. When using the spectrophotometer UV/visible, λ maximum at 280.5 nm, the reliability, assay, recovery test, and detection limits were 94.83%, 4.36%, 93.72% and 0.30 ppm, respectively. The residual styrene results obtained with this instrument was an average of 0.271%. Using variance analysis with the fixed model for all three methods (GC with the capillary column, GC with packing column, and spectrophotometer UV/visible) obtained H_0 hypothesis was rejected so that it was continued with Duncan test with the result of all three methods could be used for analysis of styrene residue. The result of average analysis for beverage sample with Spectrophotometer UV/visible method was 0.0220%, packed column GC was 0.0221% packing, and capillary column GC of 0.0.223%. For medication samples in each instruments were found 0.0239%, 0.0241%, and 0.0242%, whereas for food samples obtained 0.0236%, 0.0238%, and 0.0239%. **Conclusions:** A GC method with either a packing column or a capillary column and a spectrophotometer UV/visible could be used to analyze the residual styrene monomer of a polystyrene-grade pharmaceutical sample. The best analysis could use GC with a capillary column. It is recommended to use differential scanning calorimetry and thermogravimetric analysis, if available, as well as infrared spectrophotometer to obtain a cheaper and better method.

KEY WORDS: Gas Chromatography; Packed Column; Capillary Column; Spectrophotometer Ultraviolet/Visible; Styrene; Polystyrene

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INTRODUCTION

Plastics are simply chains of like molecules linked together. These chains are called polymers. This is why many plastics begin with "poly," such as polyethylene, polystyrene, and polypropylene. Styrene is an unsaturated aromatic monomer compound commonly used in industrial

polymer industries such as polystyrene latex and plastic making. The styrene monomer may be prepared into a polystyrene by a polymerization reaction with the aid of a catalyst. If the polymerization reaction is not perfect, then there will be unreacted monomer residues. The amount of monomer residue content depends on the purity of the material, temperature, dispersing agent, and shaking instability.[1-3] Although styrene is still considered harmless, there is a metabolic process will form styrene oxide that can cause effects that are carcinogenic.[4] According to the Food and Drug Administration (FDA), the content of styrene monomer residues in the food wrappers is 1% and 0.5% for beverage and fat packaging.[5] The dietary concentration of 2.20 ppb of styrene attributable to food packaging using the FDA's default assumption of a daily diet of 3.0 kg of food (all solids and liquids) results in an estimated daily intake of 6.6 µg/person/day (0.0066 mg/person/day).[6] Several methods have been reported in residual styrene analysis. Garrigós *et al*.,[7] in 2004, reported the use of gas chromatography–mass spectrometry (GC–MS) for their analysis, Kusch and Knupp[8] used GC and GC–MS, Ahmad and Bajahlan[9] used GC–MS, whereas Gennari *et al.*[10] used GC–flame ionization detection (FID).Headspace GC–MS was used by Amirshaghaghi *et al*.[11] Styrene peak from among 45 compounds identified in ethanol extract of *Anisochilus carnosus* in GC–MS was reported by Muthuraman *et al*.[12]

This paper reports comparison of residual styrene determination of pharmaceutical materials packed with polystyrene using GC and ultraviolet (UV)/visible spectrophotometry. So far, there is no article mentioning this comparison method. Validation method of each using GC and spectrophotometer was conducted to make sure that the method used was applicable. The comparison of the two analytical methods was analyzed statistically; then, both methods were applied to the samples obtained from the market.

MATERIALS AND METHODS

Equipment

GC Varian 3400 equipped with packed column OV101 and GC Shimadzu 17-A equipped with a DB-17 column were used.

Methods

Styrene was prepared by weighing 5 g of polystyrene latex, plus a few drops of $Al_2(SO_4)_3$ 2.5%, dried in the 500°C oven. Styrene was formed in extraction with 10 mL of methanol. This styrene extract was then analyzed by GC and spectrophotometer UV/visible.

1. Analysis of polystyrene latex sample with packed column GC: Work was done by first selecting the optimum condition of GC followed by,
   - Validation method was done using standard styrene against
   - Recurrence, accuracy, recovery, and detection limits
   - Qualitative analysis of styrene monomer residues
   - Quantitative analysis of styrene monomer residues.
2. Analysis of polystyrene latex sample with capillary column GC: Just as in the GC column packed, validation, qualitative, and quantitative analysis of the residual styrene monomer was performed
3. Analysis of polystyrene latex sample with UV/visible spectrophotometry: The procedure was performed by validating, qualitative, and quantitative analysis of the styrene monomer residues
4. Comparative analysis of residual monomer analysis
5. Application of styrene monomer residue analysis method from samples from the market.

RESULTS

The findings of the present study were recorded in Tables 1- 7 and Figure 1.

DISCUSSION

Analysis of Polystyrene Latex Sample with Packed Column GC

The results of styrene monomer analysis for the selection of optimum conditions at various temperatures are shown in Table 1. From the results obtained in Table 1, the optimum conditions were obtained at the temperature of a 200°C

Table 1: The results of styrene monomer analysis at various temperatures

Column temperature (°C)	Detector temperature (°C)	Retention time (min)	Peak area
80	200	3.746	4054
	210	3.756	4055
	220	3.768	4058
	230	3.772	4059
90	200	3.846	4068
	210	3.857	4070
	220	3.862	4074
	230	3.874	4075
100	200	4.058	4084
	210	4.062	4089
	220	4.068	4092
	230	4.078	4096
110	200	4.258	4246
	210	4.252	4189
	220	4.256	4192
	230	4.256	4192

detector, column temperature, and injector temperature, respectively, at 110°C and 200°C.

Results of Validation Method

The validation procedure was carried out based on the FDA guidances. Repeatability, precision, recovery, and detection limit were determined out of seven tests. In the regulation, it mentioned that not all of the validation characteristics were applicable for all types of tests, typical validation characteristics were specificity, linearity, accuracy, precision (repeatability, intermediate precision, and reproducibility), range, quantitation limit, and detection limit.[13,14]

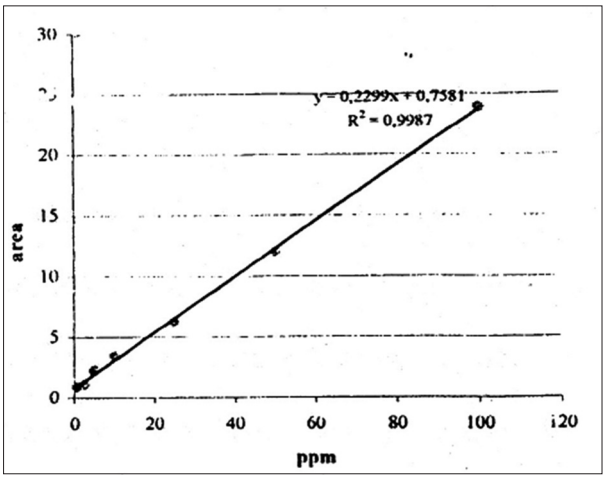

Figure 1: The calibration curve of styrene by packed gas chromatography analysis

Repeatability

Table 2 summarizes the results of six measurements using polystyrene latex samples. From this table, it can be seen that the repeatability data for GC packed capillary column method and UV/visible spectrophotometer method. It was seen that the repeatability of the residue of styrene monomer was more than 90% for the three methods used. The repeatability on the UV/visible spectrophotometer was smaller than that of GC analysis due to impurities separation that did not occur on spectrophotometers.

Precision

The precision test was performed by removing the monomer residue of styrene from the polystyrene latex sample and then adding the standard styrene monomer known to its concentration. The analytical accuracy was determined by looking at the standard percentage of the experimental standard deviation. From the observation to accuracy in the analysis of the styrene monomer residues for the three methods used are shown in Table 3. Each precision error was 2.81%, 1.29%,

Table 2: Repeatability data of styrene residue in packed, capillary column GC, and UV/visible spectrophotometry

Concentrations of residual styrene (%)			Repeatability (%)		
Packed GC	Capillary GC	Spectro UV/visible	Packed GC	Capillary GC	Spectro UV/visible
0.0148	0.0237	0.023			
0.0142	0.0239	0.0236			
0.0143	0.0238	0.023	96.67	97.88	94.83
0.0144	0.0243	0.0235			
0.0148	0.0239	0.0231			
0.0145	0.0243	0.0233			

GC: Gas chromatography, UV: Ultraviolet

Table 3: Data of precision of residual analysis of styrene monomer in packed and capillary GC and UV/visible spectrophotometer

Number of test	Styrene residue added (ppm)			Styrene concentration obtained (ppm)			Styrene average concentration			Precision fault (%)		
	Packed GC	Capillary GC	Spectro UV/visible	Packed GC	Capillary GC	Spectro UV/visible	Packed GC	Capillary GC	Spectro UV/visible	Packed GC	Capillary GC	Spectro UV/visible
1	0.5	0.5	0.5	0.4792	0.4591	0.4790						
2	0.5	0.5	0.5	0.4872	0.4920	0.4870						
3	0.5	0.5	0.5	0.4768	0.3967	0.475	0.448	0.4500	0.4470	2.81	1.29	4.36
4	0.5	0.5	0.5	0.4016	0.4971	0.3990	±	±	±			
5	0.5	0.5	0.5	0.3961	0.3922	0.3960	0.013	0.005	0.02			
6	0.5	0.5	0.5	0.4483	0.4461	0.446						

GC: Gas chromatography, UV: Ultraviolet

and 4.36% for packed GC, capillary GC, and UV/visible spectrophotometer. It could be argued that the GC capillary method was reliable enough for the analysis of the styrene monomer residue since it had obtained a fault precision of <2%. For analysis using UV/visible spectrophotometer, the precision error was <5%; it could be said that the precision of the method used was good enough and reliable. An accuracy test was not able to be done because of unavailability of material with matrix same with the sample in this research.

Recovery

The recovery test was carried out by adding a standard styrene monomer to a known polystyrene latex sample which was then reanalyzed. This test was conducted to track the residues of monomers that might be lost during the process and the efficiency of extraction. The test data for the recovery of styrene residual residues in polystyrene latex from the three methods used can be seen in Table 4. Recovery test was performed to determine the amount of residual concentration of styrene monomer lost during the extraction process. When compared to the GC method, the % retrieval obtained on the UV/visible spectrophotometric analysis was smaller, this was probably due to the absence of splitting of the impurity elements by spectrophotometry. Both packed GC and capillary GC show average recovery above 95%, so it could be said that both of these methods were quite reliable in styrene analysis in polystyrene latex. Capillary GC results showed better results due to the sensitivity of the capillary column better than the packing column.

Detection Limit

The detection limit test was carried out by diluting standard styrene monomer in methanol to obtain the smallest concentration that could still be analyzed by all three methods. The results of the limit test observations in this study found detection limits for each method of 0.25 ppm, 0.1 ppm, and 0.30 ppm for GC packed columns, capillary column GC, and UV/visible spectrophotometer.

Quantitative Analysis of Styrene

The quantitative analysis of styrene from the sample was determined based on the calibration curve describing the relationship between the peak area of the chromatogram and the concentration. Manufacture of the calibration curve was done with standard styrene solution. The analyzed sample was then plotted to the calibration curve. The calibration curve of styrene was made with a variation of 1–100 ppm concentration. An example of a calibration curve is shown in Figure 1. Making standard curves for capillary GC was done in the same way. From the curvature curve data, we could get the equation of regression line, respectively, $Y = 0.299X + 0.7581$ with $R^2 = 0.99987$ and $R = 0.9993$, $Y = 0.2328X + 1.0694$ with $R^2 = 0.9985$ and $R = 0.9992$, and $Y = 0.0322X + 0.1718$ with $R^2 = 0.9983$ and $R = 0.9991$ for packed GC, capillary GC, and UV/visible spectrophotometer. Quantitative analysis of the styrene monomer residues of polystyrene samples was carried out by three methods. This was intended to prevent the possibility of the presence of residual styrene monomers present in the solvent. The results of the residual analysis of styrene monomer from the polystyrene latex sample by the three methods used are shown in Table 5. As seen from Table 5, the residue content of styrene monomers analyzed from polystyrene latex samples was 0.217 for analysis with UV/visible spectrophotometry. This small amount was compared with the results of the GC method because of the possibility of the impurity which absence in GC gas or the presence of dimers and trimers that were difficult to avoid disturbing the absorption.

Comparative Study of Residual Styrene Monomers Methods

From the data obtained from the experiment [Table 5], then searched whether the three methods used the same or H_0 accepted. For this test, the analysis of variance with a fixed model to test the H_0 Hypothesis was applied. From the calculation results obtained data as follows [Table 6], it was found that F-arithmetic has a larger value than F-table with 95% confidence degree of 3.89% and 99% of 6.93. This showed that the Ho hypothesis was rejected which means

Table 4: Test data for residual recovery of styrene monomer in packed, capillary GC, and UV/visible spectrophotometer

Residual	Styrene	Styrene recovered (%)			Recovery (%)			Mean recovery (%)		
Styrene (%)	Added (%)	Packed	Capillary	UV/ visible	Packed	Capillary	UV/ visible	Packed	Capillary	UV/ visible
		GC	GC	Spectro	GC	GC	Spectro	GC	GC	Spectro
0.0247	0.0212	0.0446	0.0449	0.0443	94.73	95.95	93.52			
0.0247	0.0212	0.0445	0.0448	0.0449	94.33	95.55	95.95			
0.0247	0.0212	0.0455	0.0458	0.0447	98.38	99.59	95.14	96.49	97.17	93.72
0.0247	0.0212	0.0449	0.0452	0.0437	95.95	97.17	91.09			
0.0247	0.0212	0.0453	0.0456	0.044	97.57	98.79	92.3			
0.0247	0.0212	0.0454	0.0457	0.0445	97.98	99.19	94.33			

GC: Gas chromatography, UV: Ultraviolet

Table 5: Concentrations of residual styrene monomers from polystyrene samples by packed, capillary GC, and UV/visible spectrophotometry

Number of test	The residual content of styrene monomer (%)								
	Extraction			Total			Mean		
	Packed GC	Capillary GC	Spectro UV/visible	Packed GC	Capillary GC	Spectro UV/visible	Packed GC	Capillary GC	Spectro UV/visible
1	1. 0.221	1. 0.221	1. 0.226						
	2. 0.047	2. 0.049	2. 0.046	0.276	0.278	0.27			
	3. 0.008	3. 0.008	3. 0.008						
2	1. 0.221	1. 0.222	1. 0.218						
	2. 0.050	2. 0.050	2. 0.047	0.28	0.281	0.273			
	3. 0.09	3. 0.09	3. 0.009						
3	1. 0.221	1. 0.222	1. 0.217						
	2. 0.048	2. 0.048	2. 0.044	0.277	0.278	0.269	0.276	0.278	0.271
	3. 0.008	3. 0.008	3. 0.008						
4	1. 0.219	1. 0.220	1. 0.216						
	2. 0.048	2. 0.048	2. 0.047	0.274	0.275	0.271			
	3. 0.007	3. 0.007	3. 0.008						
5	1. 0.220	1. 0.221	1. 0.218						
	2. 0.046	2. 0.049	2. 0.046	0.274	0.277	0.272			
	3. 0.008	3. 0.009	3. 0.008						

GC: Gas chromatography, UV: Ultraviolet

Table 6: Data ANOVA for all three methods of analysis of residual styrene monomer

Source of variation	dB	JK	KT	F arithmetic
Mean	1	1.134375	1.134375	
Treatment	2	0.000164	0.0000632	14.04
Mistake	12	0.0000536	0.0000045	
Total	15	1.134555		

ANOVA: Analysis of variance

there were significant differences between the three methods used.[15] To determine whether the three methods used to meet the requirements of the Duncan test. From the calculation result, it was found that packed GC method, capillary GC, and UV/visible spectrophotometry can be used for the analysis of residual styrene monomers. The use of UV/visible spectrophotometry for residual analysis of styrene monomers was believed to have the same reliability as GC with 94.83% repeatability, under 5% accuracy, 93.72% recovery and can analyze styrene to the smallest concentration of 0.3 ppm. Although the two analytical methods have almost equal reliability for the best analysis, it is best to use capillary column GC as this method has better sensitivity than the two methods. Mariana[16] in her thesis experimenting with GC stated the validation of the method of analysis gave the result of the method had good selectivity, the linearity of the method with the regression equation y = 0.186x value R^2 = 0.999, the precision with relative standard deviation (RSD) = 0.93% and accuracy with percent recovery 98.04 ± 2.62% in styrene concentration added 502 µg/g, limit of detection = 0.15 µg/ L, and limit of quantification = 1.20 µg/mL.

Application to the Three Methods of Analysis on Samples Obtained from the Market

The application of the three test method was performed to test the reliability of the method for styrene analysis. This analysis was carried out by adding a certain amount of concentration known to the sample then reanalyzed. The difference in concentration was the level of the monomer residue sought. Each was done 3 times. This test was performed because no styrene monomer residue was found in various food, beverage, and medicine preparations wrapped by polystyrene. The results of this application are shown in Table 7. It could be seen that no significant concentration of styrene monomer residue was added from the added sample of 0.247% styrene. The mean analysis for beverage samples with spectrophotometric method was 0.0220%, packed GC 0.0221%, and capillary GC of 0.0233%. For medication samples in each instruments were found 0.0239%, 0.0241%, and 0.0242%, whereas for food samples obtained 0.0236%, 0.0238%, and 0.0239%. From the results of this simulation analysis, it was found that if the samples obtained on the market containing the residue of styrene monomer up to concentrations as low as 0.0247% would be able to be analyzed by all three methods. The FDA[12] requires that the residual content of styrene monomers in food wrappers is 1.0% and for packaging of beverages and fats by 0.5%.[5]

Table 7: Data analysis of residual styrene monomer from market samples by packed, capillary GC, and UV/visible spectrophotometry

Added styrene	Methods			Recovery (%)		
Concentration (%)	Packed	Capillary	Spectro	Packed	Capillary	Spectro
	GC	GC	UV/visible	GC	GC	UV/visible
0.0247	0.0213	0.0215	0.0216			
0.0247	0.0221	0.0222	0.0217			
0.0247	0.023	0.0223	0.0228			
0.0247	0.0221	0.0223	0.022	89.47	90.28	89.07
0.0247	0.0236	0.0238	0.0235			
0.0247	0.0242	0.0244	0.0239			
0.0247	0.0244	0.0244	0.0242			
0.0247	0.0241	0.0242	0.0239	97.57	97.98	96.76
0.0247	0.0236	0.0237	0.0234			
0.0247	0.0238	0.0238	0.0237			
0.0247	0.024	0.0242	0.0238			
0.0247	0.0238	0.0239	0.0236	96.36	96.76	95.55

GC: Gas chromatography, UV: Ultraviolet

Indonesian FDA[17] issued decree No. HK.00.05.55.6497 on August 20, 2007, concerning food packaging materials including polystyrene packaging used for direct contact packaging with fatty foods pH <5.0, aqueous products, water-containing products which may contain salt or sugar or an oil-in-water oil emulsion o/w or high-fat content, dairy products, non-alcoholic beverages, bread products, dry solids, with non-oil-free or fat-free surfaces, the allowed migration limits of residual styrene monomers is 10.000 ppm. Meanwhile, polystyrene packing used for direct packaging of contact with fatty foods such as water, acidic or non-acidic products, contains oils or fat-free or excess which may include a salt containing water emulsions in oils with low or high-fat content, and its derivatives, fats, and oils contain less water, bread products, the allowed total migration residual styrene monomer is 5.000 ppm. Kusch and Knupp[8] claimed a good reproducibility of the measurements with RSD values between 3.2% and 3.6% was achieved by extraction using a 75 μm carboxen-polydimethylsiloxane fiber at 60°C with 15 min sample sonication. The contents of residual styrene monomer in two samples of expanded polystyrene (EPS) were 153.2 and 65.7 mg/kg, respectively. Poouthree[18] reported their proposed method using GC–FID was able to determine residual styrene content from 50 polystyrene products bought in Thailand markets. The results from these samples showed the styrene contents at the level below 0.1%. Migration of styrene monomer, dimers, and trimers from polystyrene to food simulants was also reported by Choi *et al*.[19] Even though styrene is the danger for the body in which styrene gas can readily be absorbed through the skin and lungs,[20] styrene was reported had been used for synthesis of 1, 2-Cis-diols from olefins.[21]

CONCLUSIONS

A GC method with either a packing column or a capillary column and a spectrophotometer UV/visible could be used to analyze the residual styrene monomer of a polystyrene-grade pharmaceutical sample. The best analysis could use GC with a capillary column. It is recommended to use differential scanning calorimetry and thermogravimetric analysis, if available, as well as infrared spectrophotometer to obtain a cheaper and better method.

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