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Static Headspace GC Method: Tool for Rapid and Sensitive Analysis of Residual Solvents in Amoxicillin and Ampicillin Tablets

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Authors' contributions

This study was carried out in collaboration among all authors. Author SMH designed the study, wrote the protocol and wrote the first draft of the manuscript. Author SMZH managed the literature searches, interpreted the data and edited the manuscript. Author PS managed the experimental process and gathered data. All authors read and approved the final manuscript.

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ABSTRACT

A simple and sensitive static head space gas chromatographic (SH-GC) method equipped with FID has been developed and validated for simultaneous determination of residual solvents e.g., methanol, dichloromethane and toluene in two therapeutic drugs such as amoxicillin and ampicillin. The separation was achieved with 30 m long Elite - 5 fused silica capillary column and 0.32 mm inner diameter. The developed SH-GC method offered symmetric peak shape, good resolution and reasonable retention time for all the solvents. Beer's law was obeyed in the concentration ranges 100 – 1200, 50 – 1000 and 50 – 500 ppm for methanol, dichloromethane and toluene, respectively. The method was validated according to international conference on harmonization (ICH) guidelines in terms of specificity, linearity, precision, accuracy, limit of detection, limit of quantitation, robustness and solution stability. The degrees of linearity of the calibration curves, the percent recoveries, relative standard deviation for the method were also determined. All the validation

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parameters were within the acceptable range. The developed SH-GC method could, therefore, be suitable for simple and rapid detection of trace levels residual solvents in other pharmaceutical products and thereby it could be used for routine analysis in any analytical laboratory.

Keywords: Amoxicillin; ampicillin; residual solvents; SH-GC.

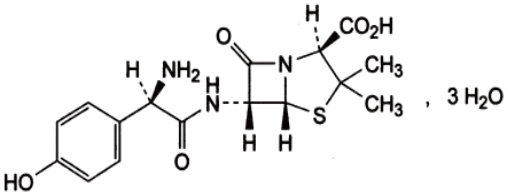
1. INTRODUCTION

The presence of solvents is essential in all steps of pharmaceutical process (e.g., reaction, separation and formulation). It is well known that a typical drug synthesis route usually consists of three to eight reaction steps and four or more different solvents are employed in the process. These solvents are not completely removed by practical manufacturing techniques and their traces may remain in the final products. The presence of these unwanted solvents (called residual solvents or organic volatile impurities) even in small amounts may influence a potential toxic risk of pharmaceutical products and have been a concern of manufacturers for many years. Moreover, residual solvents can also affect the quality and stability of not only drug substances but also drug products. Thus the amount of such solvents is limited by international conference on harmonization (ICH) guidelines [1]. Residual solvents are mainly classified into three classes on the basis of the toxicity level and the degree to which they can be considered an environmental hazard. Class I solvents are known carcinogens and are harmful to humans and the environment, so the use of these solvents should be avoided. Class II solvents are nongenotoxic animal carcinogens or possible causative agents of other irreversible toxicity such as neurotoxicity or teratogenicity. Thus these solvents should be limited in pharmaceutical products because of their inherent toxicity. Class III solvents are solvents with low toxic potential to human; no health-based exposure limit is needed. These solvents have PDEs of 50 mg or more per day. However, all the pharmaceutical products must be analyzed for residual solvents, regardless of the matrix.

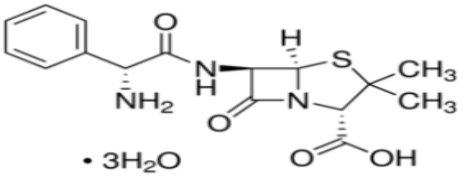
Amoxicillin and ampicillin are semi synthetic antibiotics or therapeutic drugs with a broad spectrum of bactericidal activity against many gram-positive and gram-negative microorganisms [2,3]. The structural formula of these therapeutic drugs is shown in Fig. 1. The residual solvents of these drugs have been determined quantitatively by different techniques including UV spectrophotometer [4-9], HPLC [10], HPTLC [11], RP – HPLC [12,13], fluorimetric method [14], in-vitro evaluation pH-sensitive [15] and using diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) and partial least squares (PLS) [16]. Though almost all of these systems have high selectivity and enough sensitivity, however, there still remains strong interest in finding alternative methods for detection of residual solvents of these drugs very rapidly and sensitively.

*Static h*eadspace gas chromatography (SH-GC) is the technique of choice due to its high sensitivity, excellent separation abilities, low limit of detection and simplicity of the instrumentation used for the technique. The static headspace (HS) sampling method has more appropriate sensitivity than the direct injection method because it can clearly separate volatile analytes from the sample matrix and effectively concentrate them. Therefore this method results in less complex sample preparation, decreased instrument contamination, and increased GC column life [17-21]. To our knowledge there is no validated SH-GC method available for analysis of residual solvents in commercially available tablets of augmentin (i.e., amoxicillin) and principen (i.e., ampicillin). The supplier claims that three common class II residual solvents e.g., methanol, dichloromethane, and toluene are existed in these tablets. In order to secure the safety and assure good manufacture practices (GMP), a precise quantification of residual solvents is essential.

In the present study, we report development and full validation of a novel SH-GC analytical method with FID detector for simultaneous determination of methanol, dichloromethane, and toluene. The validation was made according to ICH guidelines in terms of several parameters e.g., specificity, linearity, precision, accuracy, limit of detection, limit of quantitation, robustness and solution stability. Besides, the method is also applied to determine the residual solvents in both amoxicillin and ampicillin tablets obtained from local market. These solvents should be estimated and checked so that they may not exceed the amount specified by the ICH guidelines. The developed method is simple and



Amoxicillin Trihydrate



Ampicillin Trihydrate

Fig. 1. Structure of amoxiclllin trihydrate and ampicillin trihydrate

sensitive and could be useful for rapid routine analysis of the level of residual solvents in other drug substances.

2. EXPERIMENTAL

2.1 Chromatographic Conditions

All experiments were performed by using a Clarus 400 Gas Chromatography (GC) equipped with flame ionization detector (PerkinElmer, USA) in this study. The GC system is a microprocessor controlled gas chromatograph with an optional built – in auto sampling system. The system is also supported with additional equipment Turbo Matrix 40 Sampler. The data processing system was run with Total Chrome Navigator software which connected with PC. The solvents are separated on elite - 5 Fused silica capillary column of 30 m long & 0.32 mm in internal diameter. The column temperature is maintained at 60℃ for 5 min, then raised at a rate of 20℃ per min until 220℃ and maintained 220℃ for 5 min. The injection port and detector temperature maintained at 250℃. The carrier gas nitrogen passed with a velocity of 37.3 cm per second at 10 kpa pressure and split ratio of 20: 1. The injections, pressurized, withdraw and thermostat times are 0.1, 2.0, 0.2 and 10 minutes. The gas chromatography cycle time is only 41 minutes.

2.2 Materials

Methanol (HPLC grade), dichloromethane (LR grade), toluene (LR grade) and dimethyl sulphoxide (DMSO) (LR grade) were purchased from Ranbaxy Fine Chemicals Ltd., India. Therapautic drugs such as augmentin (i.e., amoxicillin) and principen (i.e., ampicillin) (supplier claims 250 mg each) were obtained from a pharmacy, India.

2.3 Standard Curve

In order to find calibration plots for methanol, dichloromethane and toluene, a standard stock solution of 5000 ppm was first made. For this 0.5 g of methanol, 0.5 g dichloromethane and 0.5 g toluene were mixed in 100 ml DMSO. The concentration range of 50 -2000 ppm were then made into a series of 50 mL volumetric flasks by appropriate diluting of this stock solution with DMSO. A plot of concentration (ppm) of methanol, dichloromethane, toluene in the solution (X-axis) versus peak area responses of the respective component peak (Y-axis) was drawn. The correlation coefficient (R^2), y-intercept and slope of regression line were calculated. All the peak areas were measured as a function of retention time against the diluents blank (DMSO alone).

2.4 Test Samples

Two commercially available tablets of amoxicillin (e.g., augmentin) and ampicillin (e.g., principen) (supplier claims 250 mg each) were accurately weighed and finely powdered separately. A quantity of the powder equivalent to 500 mg of each drug (either amoxicillin or ampicillin) was dissolved by shaking with 60 ml of DMSO. After passing through a 0.45 µm Millipore filter, the solution was then diluted with DMSO to obtain a concentration of about 5000 ppm. It was further diluted according to the need and then analyzed following the proposed procedures. The nominal content of the tablet/capsule was calculated either from the previously plotted calibration graphs or using regression equation.

2.5 Procedure

The HS vials have a DMSO solution containing solvents at different concentrations, the vials are kept at room temperature, and the headspace sampler was equipped with a 5-mL sample loop. We added 2 ml of sample into HS vial and tighten with septum. The turbometrix took the sample and heated in injector at injector temperature to produce vapor. Then the syringe took the vapor sample and injected into GC.

2.6 Statistics

Mean (X) was measured from at least three independent determinations for all data points. Standard deviation (SD), and relative standard deviation (RSD) were calculated in order to validity the experimental data.

3. RESULTS AND DISCUSSION

A SH-GC analytical method was developed and validated for the quantitative determination of the residual solvents of methanol, dichloromethane, and toluene in two therapeutic drug substances (e.g., amoxicillin and ampicillin). The method was validated within ICH guidelines Q2A and Q2B in terms of the following validation parameters:

3.1 Specificity

For this, methanol, dichloromethane and toluene (0.25 g each) were taken in three separate 100 ml volumetric flask and diluted with diluent, DMSO up to the mark(final concentration was 2500 ppm each). A certain amount of solution (2.0 mL each) solutions from each flask were then taken in headspace sample (HSS) vial for experiment. The results indicated that the retention times of methanol, dichloromethane, toluene and DMSO were found to be 3.1, 3.6, 6.8 and 8.2 minutes, respectively, which is shown in Fig. 2. The method showed good peak shape and narrow peak width resulted in excellent column efficiency. The blank chromatogram (due to DMSO alone) did not show any interference with the solvent peaks.

3.2 Linearity and Range

To carry out this study, different concentrations with a range of 50 -2000 ppm were prepared for each solvent. All concentrations were prepared in triplicate, by individually weighing amounts of solvents. The results were represented graphically (peak area vs concentration) to obtain calibration curves for all solvents which is shown in Fig. 3. The linearity of the calibration curves were validated by the high value of correlation coefficient (R^2 = 0.99) for all the regression equations. The plots for methanol, dichloromethane and toluene were found to be rectilinear over the range of 100 – 1200, 50 – 1000, and 50 – 500 ppm. The wide measurement range allows determination with adequate precision of different analyte contents in various matrices.

3.3 Precision

For instrument precision or repeatability six independent determinations (methanol 3000 ppm, dichloromethane 600 ppm and toluene 890 ppm) were carried out during single day and on their basis the values of relative standard deviation (RSD) were calculated. The values of RSD for methanol, dichloromethane and toluene were found to be 1.52, 0.889 and 1.105% respectively (Table 1). These values are found under acceptable limit for each residual solvent as revealed by relative standard deviation data on ICH guidelines (RSD <15.0% for the solvents).

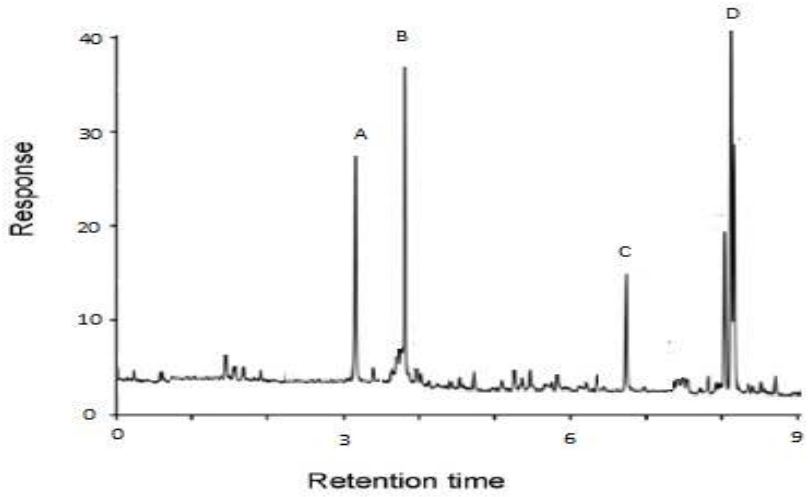


Fig. 2. Chromatogram of reference solution (Class II solvents in sample) in which residence time of methanol (A), dichloromethane (B), toluene(C) and DMSO(D) is shown

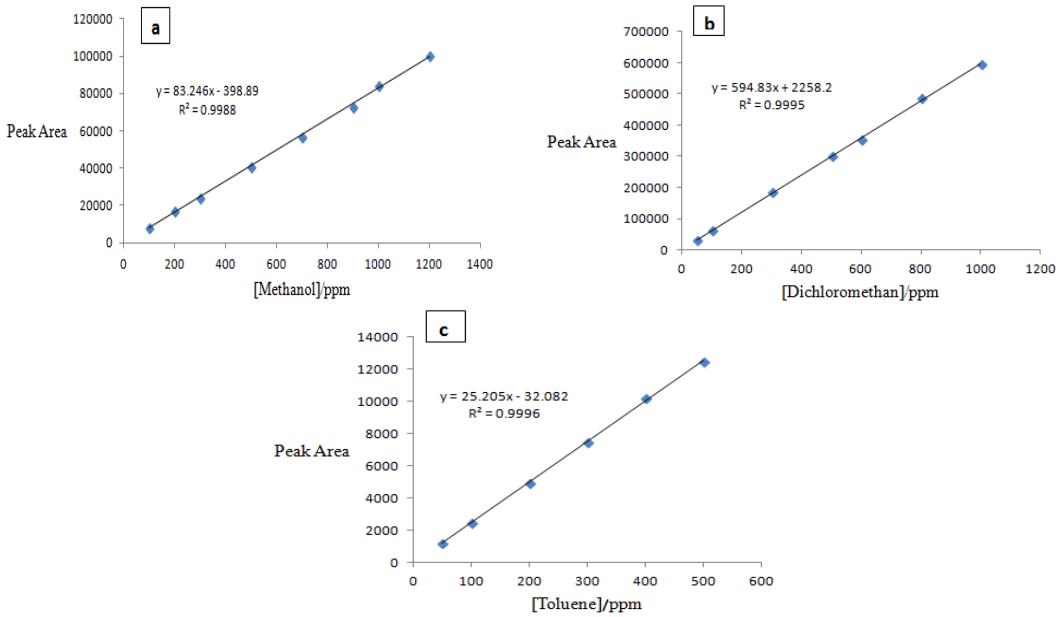


Fig. 3. Linear regressions of SH-GC determinations for the residual solvents methanol (a), dichloromethane (b) and toluene (c)

Table 1. Instrument precision or repeatability (n=6) of the determination of residual solvent at a level of methanol 3000 ppm, dichloromethane 600 ppm and toluene 890 ppm

Injection	Peak area of methanol	Peak area of dichloromethane	Peak area of toluene
Standard - 1	257852	342578	212124
Standard - 2	253654	343387	214578
Standard - 3	258614	345121	216589
Standard - 4	249856	345879	218956
Standard - 5	250128	351248	216593
Standard - 6	251452	344581	217425
Mean	256592	345465	216044
% RSD	1.52	0.889	1.105

3.4 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The limit of detection of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact amount. While the limit of quantitation is the minimum level of concentration at which the analyte can be quantified with acceptable precision and accuracy. LOD and LOQ were calculated using the signal to noise ratio (S/N) method. For LOD, a solution of 30 ppm (mixture of methanol, dichloromethane and toluene) and for LOQ, a solution of 50 ppm (mixture of methanol, dichloromethane and toluene) were prepared separately. Three replicate solutions for each case were injected into the GC and recorded. Similarly, three replicates of the blank solutions were injected and peak-to-peak noises around the retention time of each solvent were measured and subsequently signal to noise ratio calculated. The LOD and LOQ were calculated using the following equations:

$$\text{Limit of detection (Conc. ppm)} = \frac{3.3 \times TC}{S/N}$$

where,

TC = Concentration of solvent
S=Average signal of solvent in the test solution
N= Average noise of blank solution (at the same retention time of the solvent)

$$\text{Limit of quantification (Conc. ppm)} = \frac{10 \times TC}{S/N}$$

where,

TC = Concentration of solvent
S=Average signal of solvent in the test solution
N= Average noise of blank solution (at the same retention time of the solvent)

The LODs and LOQs for methanol, dichloromethane and toluene were found to be 31.05, 16.12, 15.30 and 94.1, 48.85, 46.36 ppm, respectively. The sensitivity of the method was demonstrated by low-LOD values obtained for all the solvents analyzed (Table 2).

3.5 Recovery

The recovery (%) of standard solutions for three selected samples was calculated. The standard mixture solution spiked in the sample was individually prepared as 200, 300 and 500 ppm of methanol, dichloromethane, and toluene respectively. The recovery (%) was determined for all the solvents using the following formula:

$$\%\ Recovery = \frac{\text{Concentration by correlation plot}}{\text{Actual Concentration}} \text{x } 100$$

Table 3 indicated that the recovery values of methanol, dichloromethane and toluene were found to be 98.17 – 99.97, 98.76 – 101.27 and 99.87 – 100.59 % at 200, 300 and 500 ppm respectively. The data indicates that the developed method is very accurate and precise.

3.6 Robustness

For this, three standard solutions (methanol 3000 ppm, dichloromethane 600 ppm and toluene 890 ppm) at normal analytical condition were made and experiments were conducted at varied oven temperature (experiment –A: initial oven temperature was bellow 5℃ from normal oven temperature; experiment –B: initial oven temperature was above 5℃ from normal oven temperature) in triplicate. The values of RSD for methanol, dichloromethane and toluene peak responses from three injections of standard solutions were calculated. Table 4 showed that the RSD values for methanol, dichloromethane and toluene in both experiment - A and experiment-B were found to be 0.185, 0.275, 0.037 and 1.55, 1.72 , 1.83 respectively, which is a strong agreement of ICH limits (RSD < 15 %).

3.7 Solution Stability

For this study, experiments were done after 24 hours of solution (methanol 3000 ppm, dichloromethane 600 ppm and toluene 890 ppm) preparation. The area response was recorded to calculate % RSD and the values for methanol, dichloromethane and toluene were found to be 0.569, 0.425 and 0.713 respectively (Table 5). The RSD values are acceptable of ICH limit (RSD < 15 %).

3.8 Validation of Assay Performance in Commercial Amoxicillin and Ampicillin Tablets

The developed method was applied for the determination of residual solvents in commercial tablets, namely augmentin (i.e., amoxicillin) and principen (i.e., ampicillin). The results are shown in Table 6 in which the concentration (ppm) of methanol, dichloromethane and toluene for each amoxicillin and ampicillin tablet were found to be 64.5, 2.5, 23.5 and 71.6, 2.6, 25.7 ppm respectively. These values are much lower than

Table 2. LOD and LOQ of the proposed method

Method	Injections	Peak area of methanol	Peak area of dichloromethane	Peak area of toluene
LOD (30 ppm)	Injection -1	2530	17901	765
	Injection- 2	2587	17852	758
	Injection - 3	2598	17254	753
	Mean	2571.67	17835.67	758.67
	% RSD	1.42	0.420	0.795
LOQ (50 ppm)	Injection -1	4154	28525	1152
	Injection- 2	4204	28605	1110
	Injection - 3	4120	28574	1198
	Mean	4159.33	28568	1153.33
	% RSD	1.01	0.14	3.82

Table 3. Recovery of SH-GC determinations of residual solvents for methanol, dichloromethane and toluene

Concentration	Solvent	Ave. peak area	Concentration by graph	% Recovery
200 ppm	Methanol	16245	199.93	99.97
	Dichloromethane	122734	202.54	101.27
	Toluene	5021	200.48	100.24
300 ppm	Methanol	24120	294.52	98.17
	Dichloromethane	181012	300.51	100.17
	Toluene	7520	299.62	99.87
500 ppm	Methanol	41210	495.34	99.069
	Dichloromethane	302454	493.83	98.76
	Toluene	12541	502.95	100.59

Table 4. Robustness of the proposed method

Condition	Peak area of methanol	Peak area of dichloromethane	Peak area of toluene
Oven temperature decrease 5°	256874	341254	216045
	256258	341001	215982
	255941	342741	216142
	RSD = 0.185	RSD = 0.275	RSD = 0.037
Oven temperature increase 5°	241252	335740	201458
	245360	344578	208965
	248851	346874	205214
	RSD =1.55	RSD = 1.72	RSD = 1.83

Table 5. Solution stability of the proposed method

Standard after 24 hrs	Peak area for methanol	Peak area for dichloromethane	Peak area for toluene
Injection -1	251258	345874	218045
Injection -2	252540	344103	217278
Injection-3	254125	347021	215067
Mean	252641	345666	216796
% RSD	0.569	0.425	0.713

Table 6. Determination of residual solvents in amoxicillin and ampicillin drugs

Sample	Injections	Peak area of methanol	Peak area of dichloromethane	Peak area of toluene
Amoxicillin	Injection - 1	10254	5214	1120
	Injection - 2	10210	5254	1154
	Injection - 3	10544	5287	1187
	Mean	10336	5251	1153
	% RSD	1.756	0.696	2.90
	Concentration (ppm) per tablet	64.5	2.5	23.5
Ampicillin	Injection – 1	11541	5308	1245
	Injection – 2	11408	5342	1280
	Injection – 3	11620	5405	1262
	Mean	11523	5351	1262
	% RSD	0.93	0.92	1.39
	Concentration (ppm) per tablet	71.6	2.6	25.7

their maximum ICH limits. The calculated RSD values of methanol, dichloromethane and toluene for amoxicillin and ampicillin were found to be 1.756, 0.696, 2.90 and 0.93, 0.92, 1.39

respectively which are acceptable within the limit of RSD value 15%. Thus the method is found to be applicable for routine analysis of amoxicillin and ampicillin in pharmaceutical formulations.

4. CONCLUSION

In this study, a simple, rapid and sensitive SH-GC method equipped with FID for determination of residual solvents in therapautic drugs (e.g., amoxicillin and ampicillin) was developed and validated. The method had good reproducibility and linearity for the residual solvents used in the manufacturing process. The recovery was good and justified the preparation of the standards/samples in DMSO. The % RSD values obtained in all the results were very low and within the ICH limits. Thus the present method is satisfactorily a better method for the determination of residual solvents in amoxicillin and ampicillin in both bulk and pharmaceutical formulations. It is expected that the validated method could be applied for rapid routine analysis of residual solvents for other drug substances.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. ICH guidelines for residual solvents Q3 Step 4; 1997.
2. The British Pharmacopoeia. Vol II, The stationany office, London, UK. P. 1668; 2009.
3. The British pharmacopoeia, Vol I, The stationary office, London, UK. P.149; 2009.
4. Madhura D, Vandana G, Pranavpramod J. Simulataneous estination of amoxicillin trihydrate and bromhexine hydrochloride, in oral solid dosage forms by spectrophotometer method. Int. Res. J. Pharma. 2011;2:197-201.
5. Rojanarata T, Opanasopit P, Nagawhirunpat T, Saehuan C, Wiyakrutta S, Meevootisom V. A simple, sensitive and green binzmatic uv spectrophotometric assay of amoxicillin formulations. Enz. Microb. Technol. 2010;46:292-296.
6. Nagaraja P, Shrestha A. Spectrophotometric method for the determination of drags containing phenol group by using 2,4-Dinitro Phenylhydrazine. J. Chem. 2010;7:395-402.
7. Ashik M, Kalam MA, Sultana R, Rahman E, Mahbub A, Hasnat A. Pharmacokinetic Study of amoxicillin cupsule in healthy Banagldeshi subjects using urinary excretion data, Dhaka, Univ. J. Pham, Sci. 2009;8:53-59.
8. Nojavana S, Ghassempourb A, Bashourc Y, Darbandic MK, Ahmadid SH. Determination of residual solvents and investigation of their effect on ampicillin trihydrate crystal structure. J. Pharma. Biomed. Anal. 2005;36:983–988.
9. Farajzadeh M, Mardani A. Analysis of residual solvents in ampicillin powder by headspace spectrophotometric method. Anal. Sci. 2002;18(2):171-175.
10. Dhoka M, Gawande V, Joshi P. High-preformace liquid chromotographic method for determination of amoxillin trihydrate and bromhexine hydrochloride in oral dosage forms. J. Pharm. Pharm. Sci. 2010;2:129-133.
11. Dhoka M, Gawande V, Joshi P. HPLC determination of amoxicillin trihydrate and bromhexine hydrochloride in oral solid dosage forms. J. Pham. Sci. Res. 2010;2: 447-483.
12. Nikam D, Handrakant C, Bode G, Surana S, Venkateshwarlu G, Dekate P. Development and validation of RP-HPLC method for simultaneous estimation of amoxicillin trihydrate and flucloxacillin sodium in capsule dosage form. Int. J. Pharm. Tech. Res. 2009;1:935-939.
13. Sana L, Kumar S, Bari B. Development and validation if RP-HPLC method for the simultaneous estimatin of amoxicillin trihydrate and bromhexine hydrochloride form oliy suspension, phormaceutica. Anal. Acta-open Excess. 2010;1.
14. Hui-nan T, Wei-dong G, Mou-Shengand L, Ling XY. b-Cyclodextrin enhanced fluorimetric method for determination of amoxicillin in dosage. Chem. Res. Chin. Uni. 2011;27:190-192.
15. Kumar G, Satyawansingh T. Formulation and in-vitro evaluation of P^H sensitive oil entrapped buoyant beads of amoxicillin. Inter. J. Drug Devlivery. 2011;3:125-132.
16. Parisoho G, Flores M, Joao F, Molz F. Determination of amoxicillin content in powdered pharmaceutical for mulations using DRIFTS and PLS. Braz. J. Pharm. Sci. 2007;43:89-96.

17. Haky JE, Stickney TM. Automated gas chromatographic method for the determination of residual solvents in bulk pharmaceuticals. J. Chromatogra. 1985; 321:137-144.
18. Smith ID, Waters DG. Determination of residual solvent levels in bulk pharmaceuticals by capillary gas chromatography. Analyst. 1991;116(12): 1327-1331.
19. Markovich RJ, Ong S, Rosen J. Quantitation of the residual solvent naphtha in a pharmaceutical soft gelatin capsule product by equilibrium headspace gas chromatography. J. Chromatgra. Sci. 1997;35(12):584-592.
20. Ruchatz F, Kleinebudde P, Muller BW. Residual solvents in biodegradable microparticles: Determination by a dynamic headspace gas chromatographic method. Int. J. Pharma. 1996;142(1):67-73.
21. Markovich RJ, Ong S, Rosen J. Quantitation of the residual solvent naphtha in a pharmaceutical soft gelatin capsule product by equilibrium headspace gas chromatography. J Chromatogr Sci. 1997;35(12):584-592.

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