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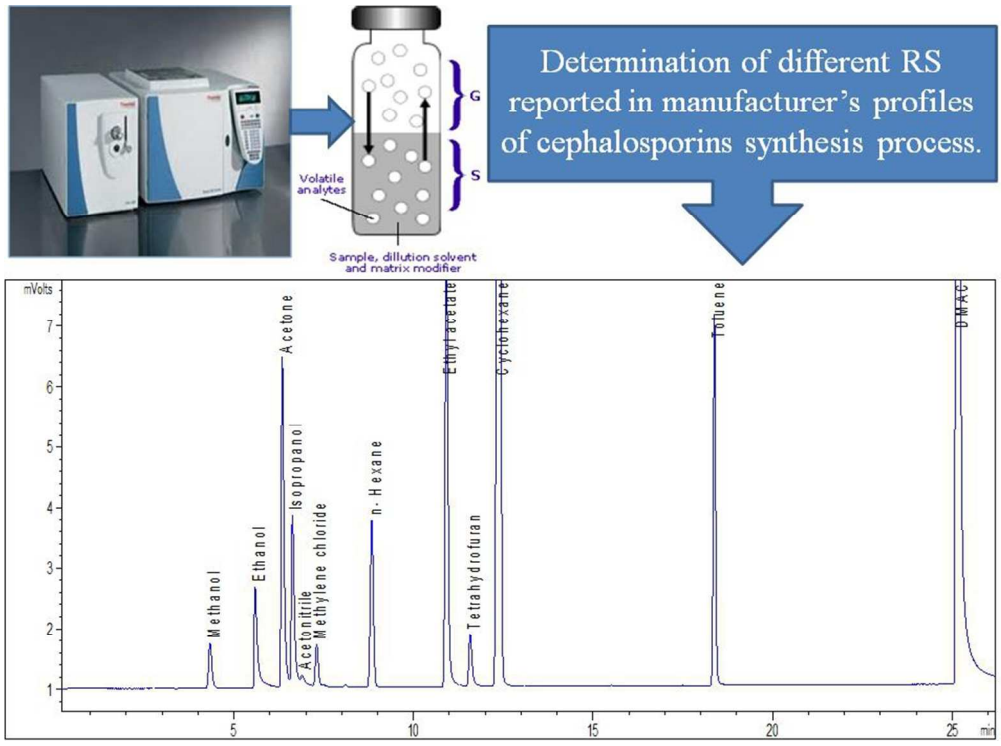

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Static Headspace Gas Chromatographic Method for Determination of Residual Solvents in Cephalosporins

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Abstract

Pharmaceutical industry is facing a daunting challenge in controlling impurities. According to GMP, measuring residual solvents is an integral part of impurity profile assessment for pharmaceutical products and is mandatory for the release testing of all active pharmaceutical ingredients or drug products according to regulatory authorities' requirements. Cephalosporins were surveyed for solvents being used in synthetic processes followed by establishment of general method for determination of those solvents. A sensitive static headspace gas chromatographic (HSGC) with flame ionization detector (FID) protocol was successfully developed and validated for determination of residual solvents commonly used in

cephalosporins synthesis. The headspace and chromatographic parameters such as split ratio, flow rate and oven programmed temperature were optimized to enhance sensitivity and chromatographic resolution. Using dimethyl-acetamide (DMA)/ water 1:1, v/v mixture as diluent, equilibration temperature of 120 °C for 5 min, programmed temperature in range of 40-155 °C, Helium as carrier gas and capillary column (6% cyanopropyl-phenyl- 94% dimethyl polysiloxane) 30 m x 0.32 mm id x 1.8µm film thickness. The proposed method was found to be suitable for determination of 11 different residual solvents, validation results had indicated method specificity, sensitivity, accuracy where recoveries ranges from 98 to 103%. Regarding all analytes r = 0.995-1.000 except for n-hexane and cyclohexane r = 0.980, 0.988 respectively.

Keywords: Residual solvents; Headspace gas chromatography; Antibiotics; Cephalosporins; Validation.

Introduction

Cephalosporins antibiotics has the structural basic nucleus shown in Figure1 **Error! Reference source not found.** (6R,7R)-3-drivative 7-acetamido derivative -8-oxo -5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid. (**Figure 1**) The therapeutic feature of these agents focused on their ß-lactam ring, which together with the adjacent atoms have spatial configuration similar to that of peptidoglycan

precursors used in the biosynthesis of bacterial cell wall [1]; they bind to enzymes called penicillin binding proteins (PBPs) resulting in inhibition of bacterial cell wall biosynthesis. Residual solvents (RS) in pharmaceuticals are organic volatile chemicals that are used or produced in the manufacture of drug substances, excipients or in formulation of drug products. A typical drug synthesis route consists of three to eight reaction steps where four or more different solvents may be employed in the process [2]. Appropriate selection of the solvent for the synthesis of drug substance may enhance the yield, or determine characteristics such as purity, particle size, crystalline structure [3], wettability [4,5], solubility, stability and dissolution properties [6,7] . Therefore, the solvent may sometimes be a critical parameter in the synthetic process. The solvents may not completely removed by practical manufacturing techniques and their traces may remain in the final product. Depending on the type/class of solvent, high levels of RS in APIs can pose a potential safety risk to patients' health due to their toxicity. Thus for toxicological and physicochemical reasons, drug manufacturers are increasingly required to monitor and limit the presence of RS in their products [8]. RS Determination is redundantly found in literature using different techniques; briefly, nonspecific techniques such as loss on drying technique [9, 10] or little advanced such as thermo-gravimetric technique [11] and determination of chlorine content for chlorinated solvents [12,13]. More sophisticated techniques such Infrared spectroscopy [14] and Fourier Transform Infrared spectrometry [15] was

used to determine residual tetrahydrofuran, dichloroethane and methylene chloride in polymer samples.

Headspace gas chromatography (HSGC) had been used since 1980s, but recently it has become a part of mainstream pharmaceutical analysis [16]. USP incorporated the technique in "<467> Residual solvents" general chapter [17]. Different headspace sampling techniques are available [18-20]: static headspace, multiple headspace extraction (MHE) and dynamic headspace extraction. A tandem headspace (HS) sampling, gas chromatography (GC) and non-selective detector like flame ionization detector (FID) or selective detector like mass spectrometry detector (MS) is the preferred technique for the analysis of volatile compounds [21, 22]. HSGC-MS was applied for the determination of RS in food [23], headspace solid-phase micro-extraction (SPME) coupled with gas chromatography and flame ionization detection was applied for the analysis of tetrahydrofuran and methanol in distillation residue samples [24]. HSGC-FID was used for the determination of residual solvents in biological fluids [22]. Also residual *n*-hexane and acetone in an active ingredient derived from beeswax was analyzed using HSGC [25]. Another chines patent had discussed the use of HSGC in determination of dimethyl acetamide and dimethyl formamide in cephalosporin medicines. Chong & Xiao-meng, had developed a method for determination of residual solvents in five cephalosporin antibiotic materials. Using SPB-1 capillary column, oven temperature of 40 °C, headspace oven was set a temperature of 70

°C for 30 min, N_2 as carrier gas [26].

There were two approaches to develop a method for residual solvents determination in cephalosporins, either establishing single method for each cephalosporin drug substance or designing a general method for all residual solvents used in their synthesis. The first approach was not used for economic reasons; consequently, second approach had been chosen. Hence, manufacturer's profiles for cephalosporins under investigation had been collected, and then studied for screening of solvents being used in synthesis process of all cephalosporins involved in the current article.

This article describes the development and validation of a general, precise, accurate and robust static HSGC method for the determination of eleven residual solvents commonly used in cephalosporin synthesis; namely methanol, ethanol, ethyl acetate, acetone, methylene chloride, IPA,THF, *n*-hexane, cyclohexane , toluene and acetonitrile in cephalosporins "Cefadroxil monohydrate, ceftazidime pentahydrate, cefalexin monohydrate, cefoperazone sodium, cefepime hydrochloride, cephradine, cefaclor monohydrate, cefazolin sodium, cefotaxime sodium, ceftriaxone sodium".

Experimental

Reagents and materials:

Purity of all reference used was more than 99% by GC, these includes; Dimethyl acetamide 99.9% (Riedel-de Haen, Hanover, Germany). Ethanol, toluene, ethyl acetate, tetrahydrofuran (prolabo, Magna Park, England). Acetone (lobachemie , Mumbai, India). Methanol, isopropanol, acetonitrile (labscan, Gliwice, Poland). Methylene chloride (Alliance Bio, Cleveland, USA). n-hexane, cyclohexane (Scharlau, Barcelona, Spain). Bidistilled water, aluminum crimp, rubber septum and 20.0 ml headspace vial (Thermo scientific, USA).

Cephalosporins drug substance samples:

All samples purity was at least 99.0 % according to the certificate of analysis. These includes; Cefadroxil monohydrate, ceftazidime pentahydrate, cefalexin monohydrate, cefoperazone sodium, cefepime hydrochloride (aurobindo pharma, Andhra Pradesh, India). Cephradine, cefaclor monohydrate (ACS Dobfar, Milan,Italy). Cefazolin sodium, cefotaxime sodium (Nectar life science, Mumbai, India), ceftriaxone sodium (Kopran, Mumbai, India), cefotaxime sodium (Biochemie, Mumbai, India).

Instruments:

Trace GC Ultra™ from thermo scientific (Waltham, USA) equipped with flame ionization detector (FID), split-splitless injector (SSL), Triplus™ autosampler static headspace, G43 capillary column (6% cyanopropyl-phenyl - 94% dimethyl polysiloxane) 30 m x 0.32 mm id x 1.8 µm film thickness, Helium as carrier gas, data manipulation software Chemistation™.

Method and Chromatographic Condition:

Headspace GC instrumental conditions:

The optimized parameters of HSGC-FID instrument are reported in Table 1.

(Insert Table 1)

Standard and sample preparation

Stock standard solution:

Volume of each reference standard solvent illustrated in Table 2 was transferred quantitatively into a clean dry 250.0 mL volumetric flask that contained 50 mL dimethyl acetamide then the volume was completed using dimethyl acetamide as diluents.

(Insert Table 2)

Working standard preparation:

One milliliter was transferred quantitatively from stock standard into 200.0 mL volumetric flask and the volume was completed using dimethyl acetamide.

Application of standard to HS vial:

One milliliter from working standard solution is transferred quantitatively into headspace vial that contained 1.0 mL water then the vial was immediately sealed with rubber septum and aluminum crimp then applied to HSGC-FID instrument, adopt only one injection per vial.

Sample preparation:

Approximately 100 mg of cephalosporins was accurately weighed and transferred into a 20 mL headspace vial followed by addition of water (1.0 mL) and of DMAC(1.0 mL). Cephalosporins were completely dissolves in the diluent/solvent. Then vial was immediately sealed with rubber closure and aluminum crimp, and then homogenized using vortex mixer, adopt only one injection per vial to be adopt. The vial was loaded into the headspace oven and equilibrated at 120 0C for 5 min. 2.0 mL of the resulting headspace sample was injected into the GC system via a 2.5 mL gas tight syringe.

Caution: HSGC instrument utilizes high temperature and compressed gasses. Therefore, suitable safety precautions should be taken.

Calculation:

Amount of each solvent in test solution in [ppm] was calculated using the next formula:

$$\mathbf{Content_i}(ppm) = \frac{P_i}{P_{st} - P_i} \times \frac{V_i(ml)}{W_t(g)} \times Activity_i \times Density_i\,(g/ml) \times 20$$

Where

Pi : peak area of test residual solvent. *Pst* : peak area of standard residual solvent. *Wt* : test weight. *Vi* : residual solvent *I* volume in standard. Densityi : the density of residual solvent. Activity*i* : activity percent of solvent. i = analyte

Results and Discussion:

According to the International Conference on Harmonization (ICH) guidelines [26, 28- 29] RS are divided into four different categories starting from the most toxic solvents, and ending by solvents with insignificant human toxicological effect. Excellent sensitivity and high selectivity of GC for volatile compounds makes it one of the most practical and popular techniques to determine RS in bulk APIs. In last decade, sampling techniques using static headspace gas

chromatography (HSGC) gained preference and popularity over the direct injection GC because of various complications and disadvantages caused by the direct injection of the API into the GC system [30]. HSGC are superior to direct injection GC due to minimization any potential interference caused by non-volatile substances. In contrast to HSGC, direct injection method requires relatively high sample concentration for good sensitivity; while in HSGC adjusting equilibration conditions and sample diluent dramatically affect sensitivity even if sample concentration is low. Different headspace sampling techniques are available: static headspace, multiple headspace extraction (MHE) and dynamic headspace extraction. Static headspace extraction has advantages compared to multiple headspace extraction method, which require extensive and lengthy method development procedures. In addition, it is more easily automated for the analysis of a large number of samples in a timely fashion, dynamic headspace methods often are not readily automated and require repeated cleaning of fragile glassware [18, 31].

USP general chapter "<467> Residual solvents" [17] deals with general methods for determination of residual solvents in drug substance or drug products. USP utilizes nonspecific procedures like loss on drying for class 3 up to HSGC as limit test for class 1 and class 2. Those techniques are nonspecific for cephalosporins since it deals with many solvents that have no use in cephalosporins synthesis

processes. Additionally the procedure described by the USP required 45 – 60 min for sample equilibration and >60 min for GC analysis which may cause thermal decomposition due to long equilibration times when applied to cephalosporins. Furthermore, USP issued different procedures according to aqueous solubility of RS. Thus, it seems worthy to develop a validated HSGC method for the determination of different RS reported in manufacturer's profiles of cephalosporins synthesis processes. The proposed method has the advantage of overcoming solubility problems with respect to all analytes by using DMAC/water 1:1, v/v as solvent mixture that allow the analysis of 11 solvents namely; methanol, ethanol, ethyl acetate, acetone, methylene chloride, IPA,THF, n-hexane, cyclohexane , toluene and acetonitrile in a single run. Moreover, the proposed method utilizes only 5.0 minutes for sample equilibration which overcome thermal decomposition of cephalosporins and less than 30.0 min for GC analysis.

Headspace method optimization:

Critical elements of a new HSGC method development are: (i) Identifying an appropriate diluent. (ii) Determining suitable headspace parameters (i.e., headspace equilibration temperature, time, agitation frequency), GC parameters (i.e., inlet split ratio, inlet temperature) and GC temperature programming to improve the sensitivity, selectivity of the method.

Matrix selection

Diluent selection rational is to provide solubilization of all analytes; also it should provide suitable activity coefficient (γ_i) (as large as possible) to reduce partition coefficient for analytes in system [32] in addition to have relatively high boiling point, availability, high purity and thermal stability. Different solvents were investigated such as water, DMSO, DMF, DMAC, DMAC/Water mixture. The use of water results in solubility problems with some analytes in addition to its relatively low boiling point. Although, good thermal stability provided by DMSO in addition to its high purity and high boiling point but solubility difficulties with some analytes was found. Eventually, DMF was the first solvent that satisfies properties of required solvent but for internal policy it was not selected. DMAC was the solvent of choice as it has relatively high boiling point, thermal stability, high purity, and high solublizing capacity for all RS under study. Most analytes possess higher solubility in DMAC than do in water hence the partition coefficient in DMAC is much higher. Upon addition of water to the system the solubility decreases and hence the partition coefficient decrease lead to increase the headspace concentration of analytes i.e., increasing the activity coefficient (γ) result in increasing in the concentration of the headspace for most analytes.

So mixture of DMAC/Water 1:1, v/v was chosen as diluent for final step of sample preparation (application of standard or sample to HS vial) to improve sensitivity and enhance solubility of wide variety of cephalosporins without affecting viscosity of resultant solution.

Mixing of water and DMAC was found to be exothermic so care must be taken to ensure no loss of analytes upon application of standard or sample to HS vial, through immediate sealing of vial upon addition of standard solution to water or DMAC to water and sample in case of sample application to HS vial.

Phase ratio (β) effect on HS concentration

Phase ratio (β)—ratio between volume of gas phase to that of condensed phase (31). Different filling volumes of 1.5, 2.0, 2.5, 3.0 mL correspond to phase ratio (β) of 12.3, 9, 7, 5.7 had been investigated to clarify the phase ratio effect on the headspace concentrations of analytes with different partition coefficients. Peak area (A) and concentration of analyte in headspace (C_G) are related to initial concentration of analyte (C_o), partition coefficient (K) and (β) as indicated in equation $A \propto \left(C_G = \frac{C_O}{K+\beta}\right)$. Figure 2, shows that in most cases phase ratio has minimal impact on headspace concentration of analytes indicating high K relative to β, hence headspace concentration is dominated by changes in K not β. In other words, sample volume has minor effect on analytes concentration in gas phase. It is advantageous to have response robust for the error in the sample volume since

sample volume is subjected to different personal and glassware variations. Phase ratio of 9.0 had been selected to be the optimum, i.e., 2.0 mL were selected as filling volume into 20.0 mL headspace vial to be the optimum filling volume.

(Insert Figure 2)

Equilibration time effect on HS concentration

Too short equilibration time will result in incomplete equilibration and poor headspace precision while excessive equilibration time result in thermal decomposition of drug. Thus, equilibration times of 5.0, 10, 15 and 20 minutes were investigated as illustrated in Figure 3. It was obvious that upon prolonged equilibration for more than 5.0 minutes; the solution turns to darkish yellow then black and the pressure markedly increase within the headspace vial indicating thermal decomposition and evolution of gaseous degradation products. Therefore, 5.0 minutes was chosen as optimum time for equilibration combined with agitation that enhances faster equilibration, consequently sufficient precision according to concentration level and HSGC technique.

(Insert Figure 3)

Equilibration temperature effect on HS concentration

Equilibration temperature is the most critical parameter in current work that can

jeopardize precision, as it is exponentially related to pure vapor pressure of analytes [32], consequently increasing temperature result in decreasing partition coefficient and increasing headspace concentration of analytes all in nonlinear mode. As shown in **Figure 4**

(Insert Figure 4), at any temperature the slopes of curves greatly differ consequently controlling this parameter is of great importance. Some analytes show lower HS concentration upon increasing equilibration temperature, which may be interpreted by change the diluent composition ratio. The solvent mixture composition ratio is markedly changed by changing equilibration temperature especially over 100 °C; after equilibration the initial composition of solvent mixture (DMAC: Water = 1:1) no more retained, but it will be richer in DMAC than water due to water evaporation into headspace. The decline in headspace concentration for some analytes (Figure 4) like (n-hexane, methylene chloride, cyclohexane, toluene, ethyl acetate, tetrahydrofuran) could be attributed to the increase of their activity coefficient (γ_i) in solvent mixture due to evaporation of water which lends the condensed phase (solvent mixture) richer in DMAC. In other words, at equilibration temperature greater than 100 °C those analytes show greater affinity toward the solvent mixture (richer in DMAC) than the initial solvent mixture (DMAC: Water =1:1). Temperature of 120 °C provides a satisfactory headspace concentration of most analytes and hence satisfactory sensitivity without thermal degradation.

(Insert Figure 4)

Chromatographic optimization and Carrier gas

Helium was selected as the most appropriate carrier gas based on van Demeter relation of linear velocity and height equivalent to theoretical plate (HETP) [33]. Helium provides wide range of liner velocities while maintain HETP nearly constant. Liner velocity of 24 cm/sec was found to be associated with relatively flat range of HETP thus provide a high velocity without significant loss of resolution or peak properties.

Retention of analyte is inversely related to the analyte vapor pressure (P_i^o); according to Clausius- Clapeyron equation [34]; $\log(P_i^o) = \frac{-\Delta H}{2.3\,RT} + const.$ elevating temperature (T) logarithmically increase (P_i^o). In other words, increasing temperature lead to sizable increase in the vapor pressure and less time analyte spend in the stationary phase leading to shorter retention time. Isothermal temperature conditions are used for solutes with similar retention, which is not the case we deal with. Due to wide boiling points range 39-110°C of the analytes. A programed temperature gas chromatography (PTGC) involves heating the oven at a controlled rate during the run. This allows the faster analysis of analytes with dissimilar retention; at the same time minimize peak broadening with an increase in retention. Oven temperature programs with different slopes and ramps, initial temperature and initial temperature hold time had been investigated to optimize temperature program. Final HSGC method presented in this article are listed

. In addition, **Figure 5** shows typical standard solution chromatogram indicating adequate resolution between different residual solvents peaks.

Application of purposed method on different cephalosporins samples reveled existence of some residual solvents as shown in **Figures 6,7**. Cefadroxil, ceftriaxone, cefalexin, ceftazedime and cephradin show clear chromatogram consequently revel efficient residual solvents removal; cefaclor shows residual methylene chloride and ethyl acetate; cefepim shows residual acetone and isopropanol; cefotaxime shows residual ethyl acetate; while cefoperazone, show residual acetone, ethyl acetate; cefazolin show residual acetone. Nevertheless, the detected residuals solvents were within acceptable ICH limits.

(Insert Figures 5-7)

Method validation:

Method was validated according to ICH guidelines [35] regarding specificity, linearity, precision, accuracy, limit of detection (LOD), and limit of quantitation (LOQ).

Specificity:

Headspace sampling technique contributes greatly to selectivity as it eliminates the drug matrix and only introduces volatile sample components to be analyzed

via GC. Specificity was indicated via retention time, and standard spiking approach [36]. application of proposed method on different cephalosporins namely "Cefaclor, cefadroxil, ceftriaxone, cefalexin, ceftazedime, cefepime, cefazolin, cefotaxime, cefoperazone" indicated absence of interference with RS peaks and adequate resolution between peaks (>1.5). Only for ceftazedime an unidentified peak partially overlaps toluene peak but still acceptable resolution exist.

Linearity:

Linearity was investigated using five concentration levels starting from 50 % of standard solution up to 150% with intervals of 25%. An unweighted linear regression was constructed for each analyte, slope and intercept, standard error of regression line (Syx), correlation coefficient (r), LOD, and LOQ are reported in Table 3. LOD, LOQ were driven from residual standard deviation of the regression line [35] according to equations "$LOD = \frac{3.3\sigma}{S}$ and $LOQ = \frac{10\sigma}{S}$". Where σ is the standard deviation of regression line and S is the slope of the regression line.

(Insert Table 3)

Precision:

Precision was investigated over three stages "Repeatability, Intermediate precision, Reproducibility" six different standards were prepared, filled in headspace vials on duplicate basis then chromatographed for each stage. According to the National Health Surveillance Agency (ANVISA–Brazil) [37]

and Mark B., Joachim E [38] the relative standard deviation (RSD) for the determination of precision should not exceed 15%. RSD of all solvents were found to be below 15% for each of three levels. Additionally a one-way analysis of variance (One-way ANOVA) had been used to tests the hypothesis that the means of several populations (Repeatability, Intermediate precision, Reproducibility) are non-significantly different regarding all analytes. One-way ANOVA indicated at "α= 0.05" non significantly difference, Results are reported in Table 4.

Accuracy:

Triplicate standard solutions over three concentration levels namely 75%, 100% and 125% of targeted standard concentration had been chromatographed on duplicate (two HS vials) basis, and the recovery percentage had been calculated. One sample t-test was carried out to test that true mean equals 100.0%. Regarding all analytes illustrated in Table 4 the calculated t-value for all analytes does not exceed critical t-value consequently the true mean is not significantly differ from 100.0%. Consequently, conclude method accuracy.

Application on cephalosporins samples:

Regarding recovery percentage acceptance criteria, residual solvents can be treated in the same way as minor components [39]. Consequently, acceptance limit for HSGC determination of residual solvents accuracy is 80-120 %

[31,39,22]. It was hard to get sample free from residual solvents so "Cefaclor, cefadroxil, ceftriaxone, cefalexin, ceftazedime, cefepime, cefazolin, cefotaxime, cefoperazone" samples were chromatographed with and without spiking with standard solution. The recovery range for samples was found to fall in range 80-120 % as illustrated in Table 4.

(Insert Table 4)

Conclusion:

A general and simple HSGC method was successfully developed and validated for the qualitative and quantitative determination of residual solvents in cephalosporins. Superior to USP method this method has been shown to determine different RS reported in manufacturer's profiles of cephalosporins synthesis processes. The proposed method has the advantage of overcoming the solubility problems with respect to all analytes by using DMAC/water 1:1 as solvent mixture that allows analysis of eleven solvents namely; methanol, ethanol, ethyl acetate, acetone, methylene chloride, IPA, THF, n-hexane, cyclohexane , toluene and acetonitrile. Moreover, the method utilizes only 5.0 minutes for sample equilibration, which overcome thermal decomposition of cephalosporins, and less than 30.0 min for GC analysis. Thus, it could be applied for routine work in quality control for cephalosporins antibiotics as a specific group shares certain types of solvents being used in their synthetic processes.

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Table **1**: HSGC, temperature program optimized parameters.

Parameter	Optimized value
Headspace	
Equilibration temperature [°C]	120
Equilibration time [min]	5
Phase ratio	9
Agitation frequency [sec]	30 on / 20 off
Syringe temperature [°C]	120
Injection volume [ml]	2
Injector	
Base Temperature [°C]	150
Split ratio	1:9
GC	
Flow rate [cm/sec]	24
Carrier gas	Helium
Oven temperature ramp 1	Temperature held at 40 °C for 2 min
Oven temperature ramp 2	Temperature raised to 50 °C at 2.5 °C /min then held constant for 3 min
Oven temperature ramp 3	Temperature raised to 155 °C at 5.0 °C /min
FID Detector	
Air/H_2 flow [ml/min]	350/30
Temperature [°C]	250

Table 2: Reference standard volumes

Residual solvent	**Volume [mL]**
Methanol	20
Ethanol	30
Acetone	30
Ethyl acetate	30
Methylene chloride	3
IPA	30
THF	3
Toluene	5
Cyclohexane	25
n-Hexane	2
Acetonitrile	3

Table 3: Validation parameters; Regression, LOD and LOQ results

Residual solvent	**Slope** [a]	**Intercept** [a]	**$S_{y,x}(\sigma)$** [a]	**r** [a]	**LOD (ppm)**	**LOQ (ppm)**
Methanol	0.032	0.777	0.205	0.999	20.955	63.500
Ethanol	0.056	0.457	0.320	1.000	18.939	57.391
Acetone	0.136	-3.723	2.271	0.997	55.141	167.095
IPA	0.061	-1.020	0.467	0.999	25.198	76.358
Acetonitrile	0.042	-0.328	0.049	0.998	3.835	11.621
Methylene chloride	0.090	-0.243	0.298	0.996	10.952	33.187
N-Hexane	0.989	-4.443	2.399	0.980	8.003	24.251
Ethyl acetate	0.165	-3.020	3.649	0.996	73.020	221.274
THF	0.192	-0.553	0.448	0.996	7.684	23.285
Cyclohexane	1.048	-49.983	28.466	0.988	89.649	271.663
Toluene	0.654	-2.470	2.465	0.995	12.433	37.675

[a] Calculations was done using Minitab®16.1.1 statistical package software.

Table 4: ANOVA results for comparison among repeatability, intermediate precision, and reproducibility, accuracy results showing mean recovery, 95 % confidence interval and t-value, sample recovery percentage.

Residual solvent	ANOVA *F*-value[a]	Accuracy			Sample Recovery %
		Mean	95% CI	t-value[b]	
Methanol	2.62	98.79	(95.60, 101.97)	-0.88	96 -117
Ethanol	0.85	98.77	(95.55, 101.99)	-0.88	89 -108
Acetone	0.59	100.4	(96.45, 104.39)	0.24	85 -106
IPA	0.53	99.69	(96.39, 102.99)	-0.22	87 -101
Acetonitrile	2.88	99.39	(93.27, 105.51)	-0.23	95 -111
Methylene chloride	1.09	99.48	(94.32, 104.65)	-0.23	82 -94
N-Hexane	2.25	103.7	(91.97, 115.44)	0.73	80 -98
Ethyl acetate	0.25	100.8	(96.50, 105.18)	0.45	86 -97
THF	0.24	100.6	(96.61, 104.61)	0.35	84 -101
Cyclohexane	2.53	102	(93.13, 110.89)	0.52	83 -98
Toluene	0.93	100.8	(96.18, 105.43)	0.4	85 -99

[a]Critical *F*-value $_{(2,29,0.05)}$=3.33

[b]Critical *t*-value$_{(8,0.05)}$= 2.306

Figures

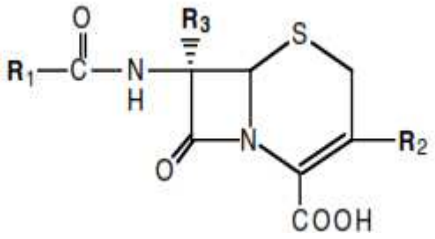


Figure 1. Cephalosporins basic nucleus

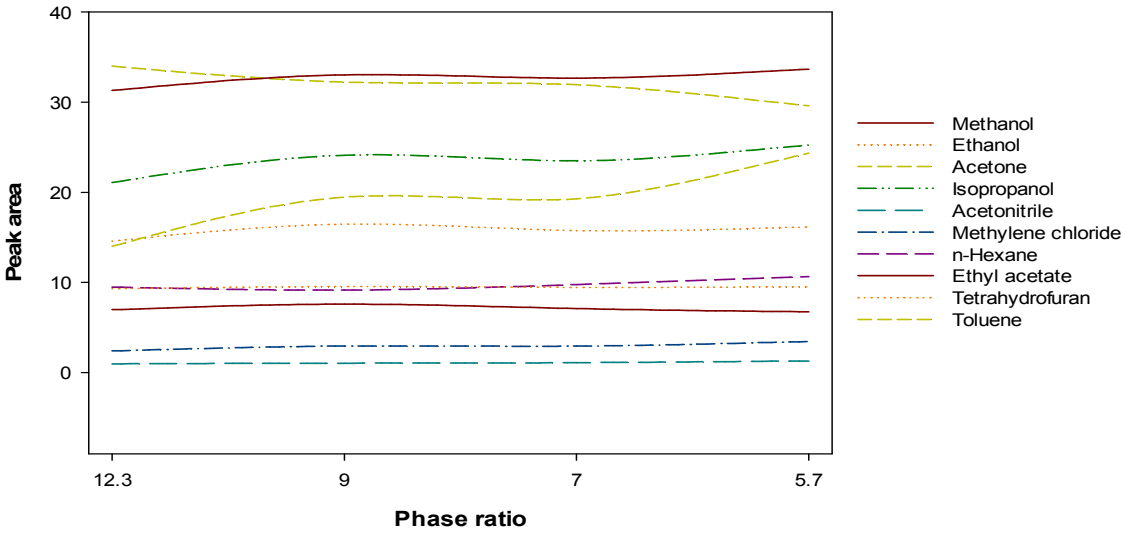


Figure 2: Relation between peak area of different RS and Phase ratio

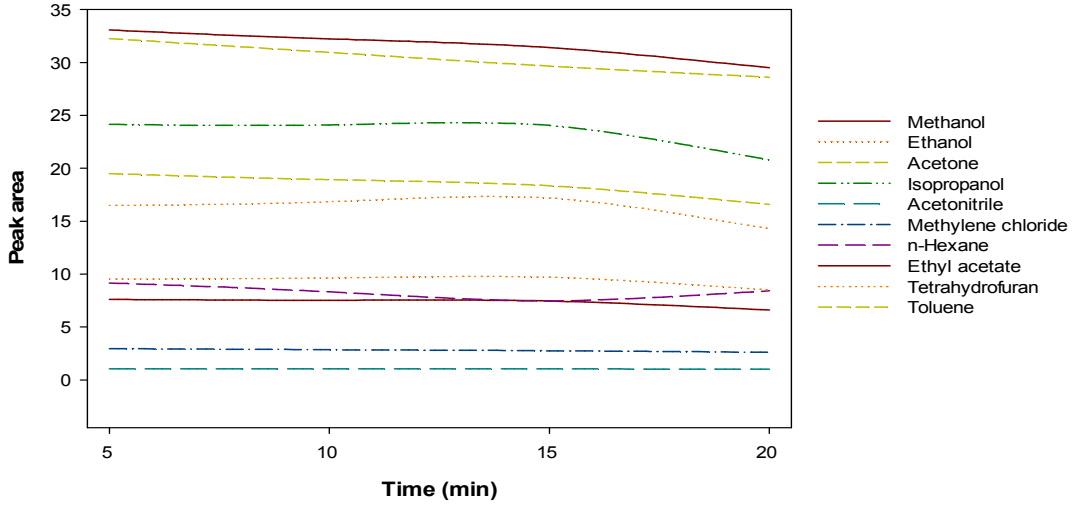


Figure 3: Relation between peak area of different RS and headspace equilibration time

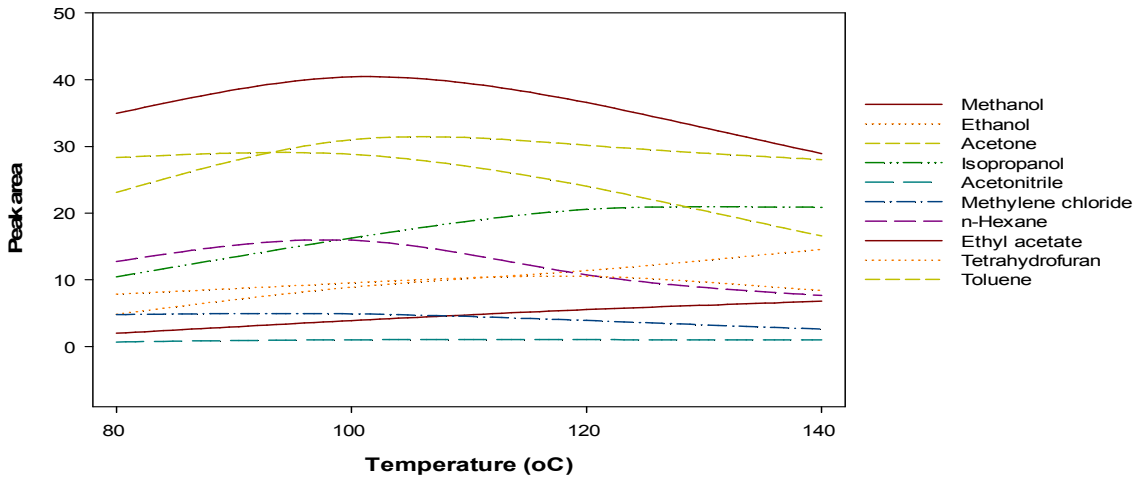


Figure 4: Relation between peak area of different RS and headspace equilibration temperature

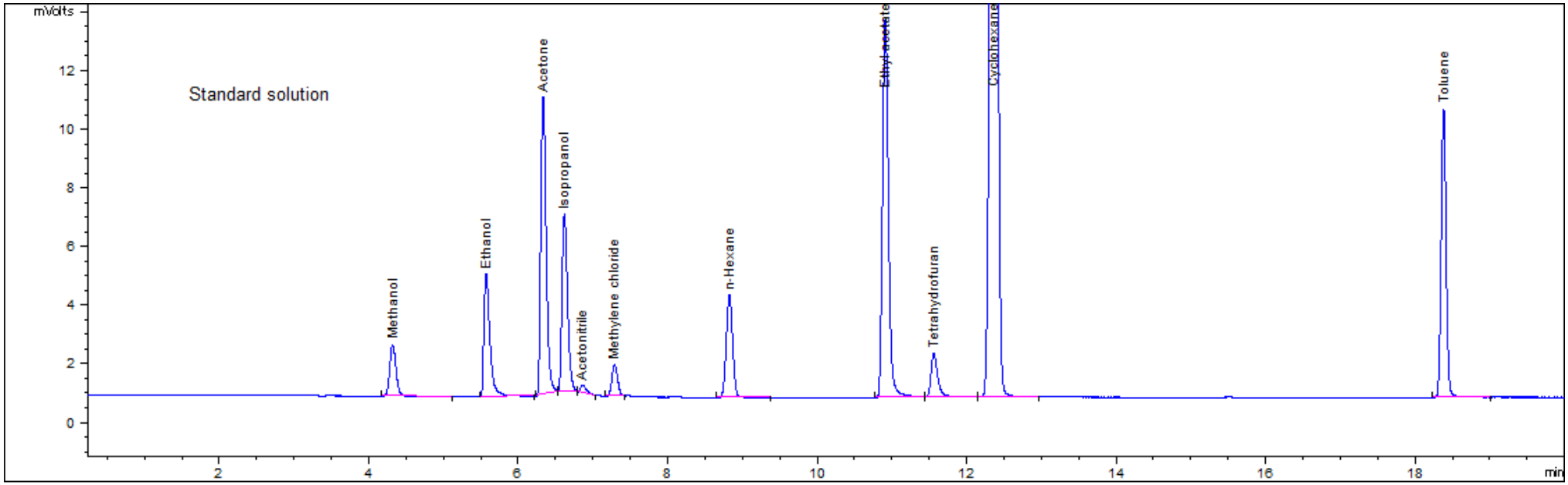


Figure 5: HSGC-FID Residual solvents typical chromatogram

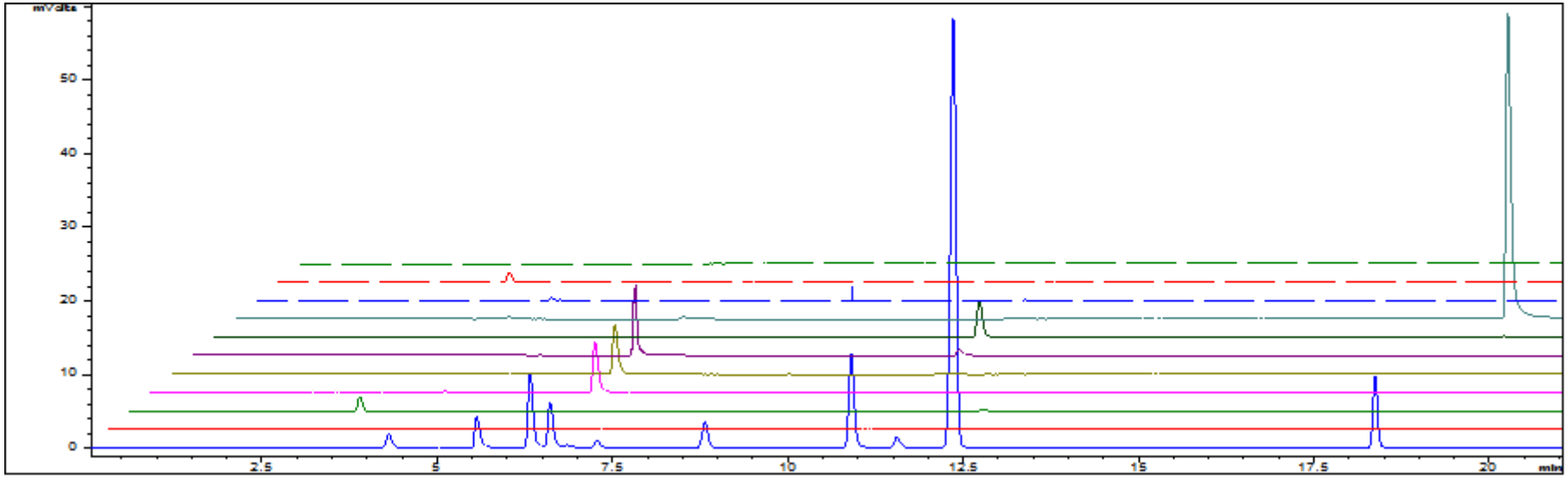
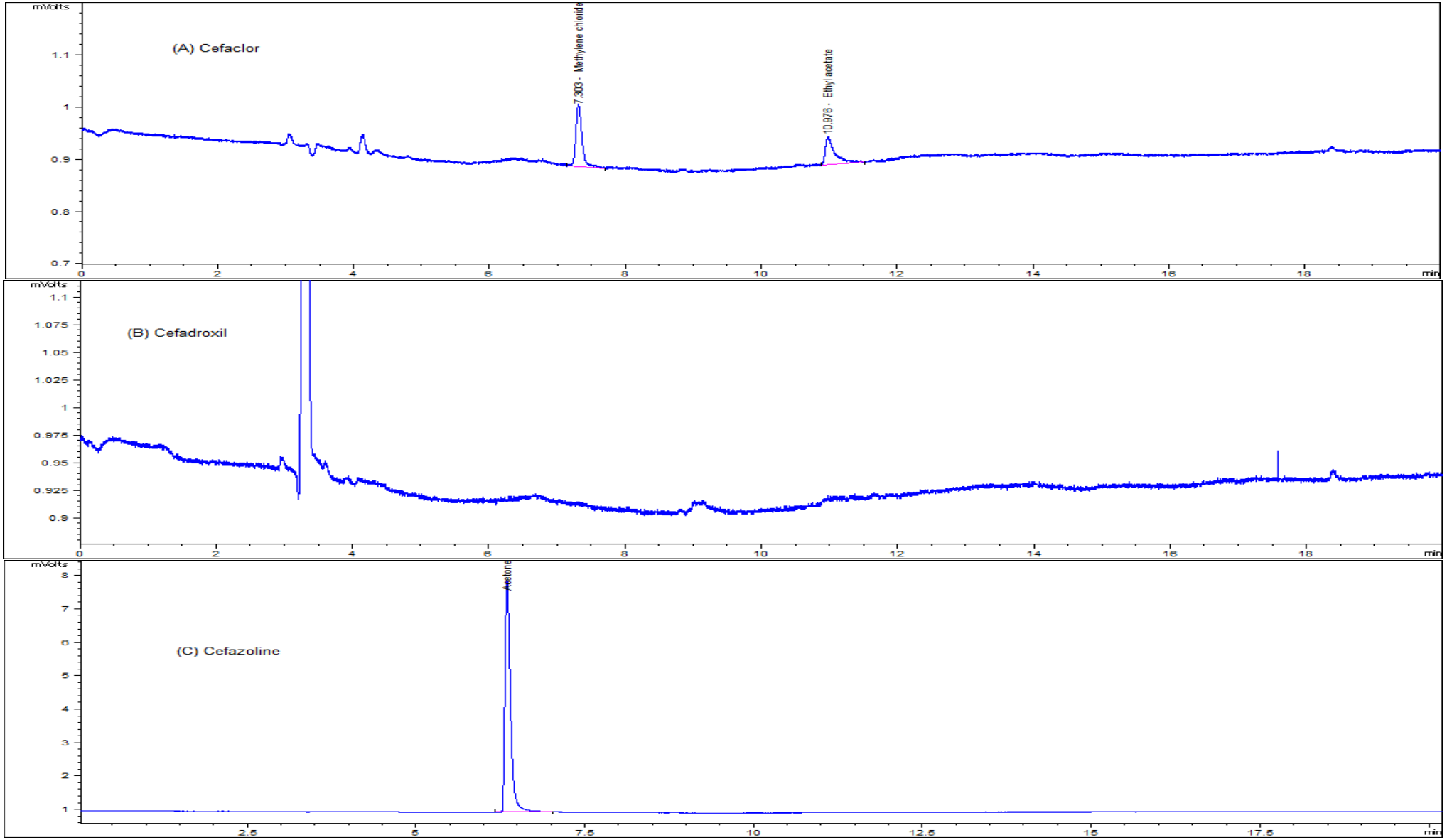
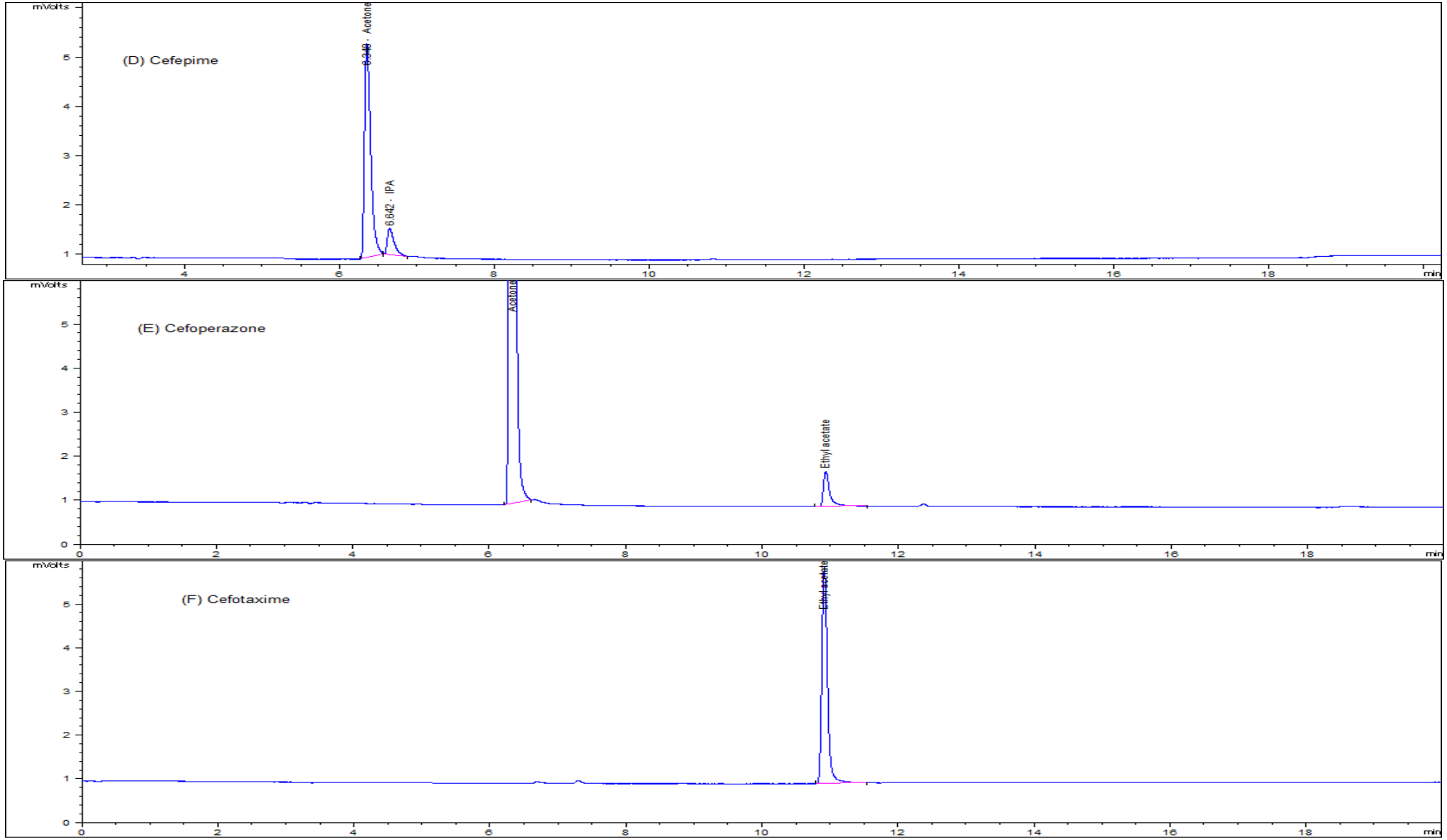
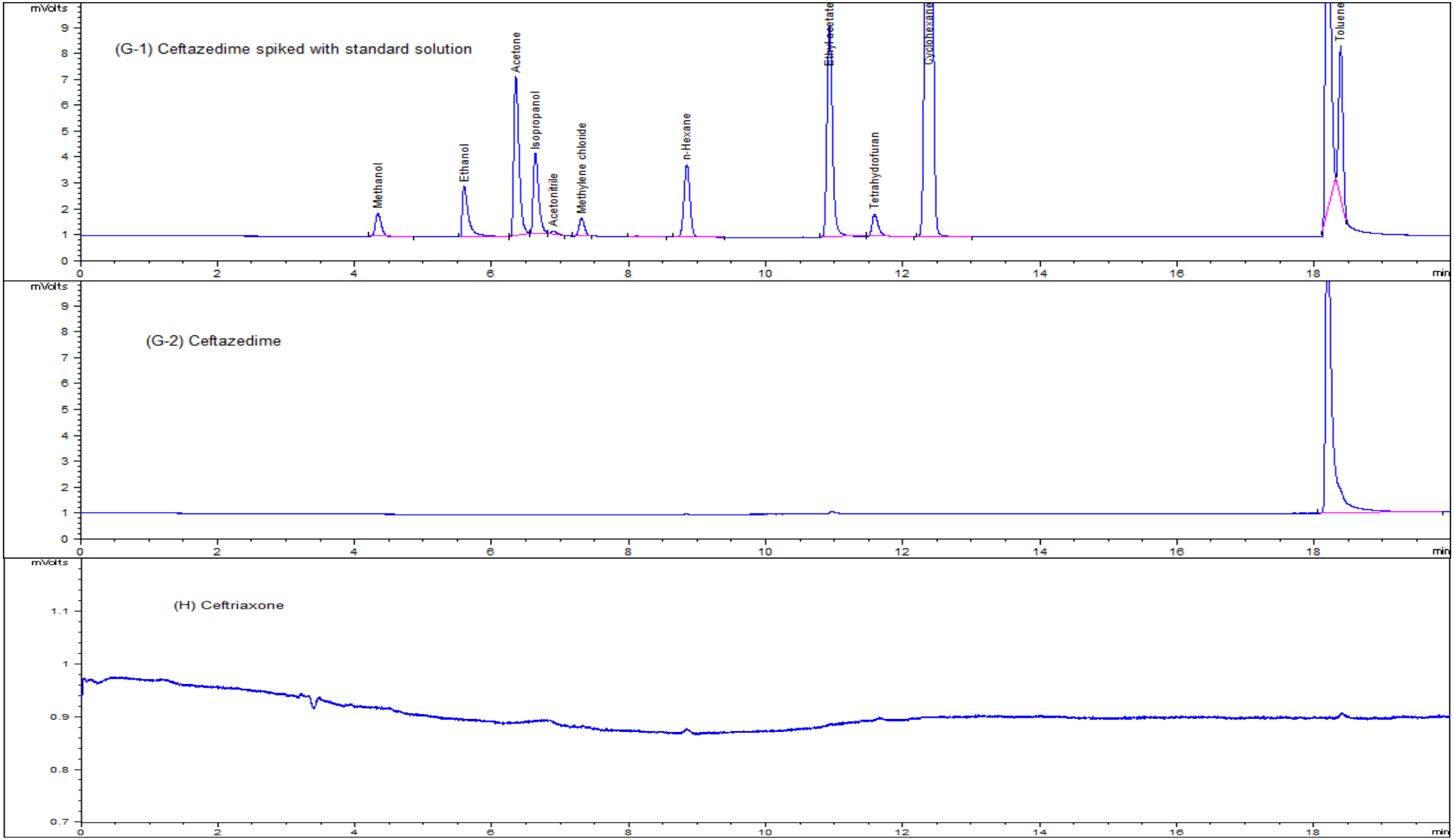


Figure 6: Overlapped chromatograms showing from bottom to top RS standard solution, cefaclor, cefadroxil, cefazolin, cefepim , cefoperazone, cefotaxime, ceftazedime, ceftriaxone, cefalexin, cephradin.


mVolts
(A) Cefaclor
7.303 - Methylene chloride
10.976 - Ethyl acetate
min
(B) Cefadroxil
(C) Cefazoline
Acetone


mVolts
(D) Cefepime
6.642 - IPA
min
mVolts
(E) Cefoperazone
Acetone
Ethyl acetate
min
mVolts
(F) Cefotaxime
min


mVolts
(G-1) Ceftazedime spiked with standard solution
Methanol
Ethanol
Acetone
Isopropanol
Acetonitrile
Methylene chloride
n-Hexane
Ethyl acetate
Tetrahydrofuran
Cyclohexane
Toluene
min
(G-2) Ceftazedime
(H) Ceftriaxone

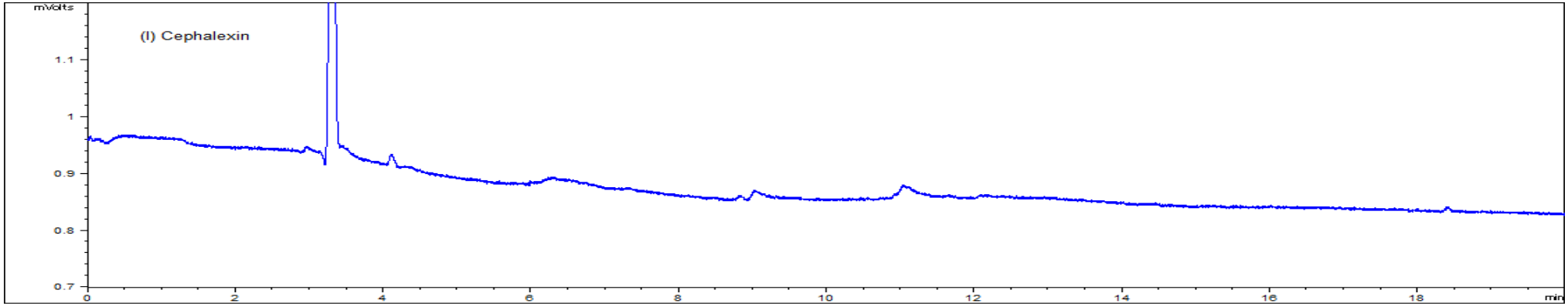


Figure 7: GC-FID Chromatograms indicate residual solvents content for (A) Cefaclor ”Contains methylene chloride and ethyl acetate”, (B) Cefadroxil ”residual solvents free”, (C) Cefazolin ”contains Residual acetone”, (D) Cefepime “contains residual acetone and isopropanol”, (E) cefoperazone “contains residual acetone,ethyl acetate”, (F) Cefotaxime “contains residual Ethyl acetate”, (G) Ceftazedime “standard spiked sample (G-1) chromatogram show partially overlapping unidentified peak close to toluene and sample (G-2) contains the same unidentified peak indicating absence of residual toluene in sample”, (H) Ceftriaxone ”residual solvents free”, (I) Cefalexin ”residual solvents free”.