การพัฒนาวิธีวิเคราะห์ยากลุ่ม cephalosporins โดยใช้ HPLC สำหรับหาคุณภาพยาที่มีจำหน่ายในท้องตลาด

Analytical Method Development Using HPLC for Investigation of the Quality of the Marketed Cephalosporins

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บทคัดย่อ

วัฏภาคเคลื่อนที่ที่เหมาะสมสำหรับการวิเคราะห์ยากลุ่ม cephalosporins โดยใช้คอลัมน์ C_{18} ชนิด Hypersil ขนาดอนุภาค 5 ไมโครเมตร เส้นผ่าศูนย์กลาง 3.9 มิลลิเมตร ความยาว 250 มิลลิเมตร คือ ส่วนผสมระหว่าง acetonitrile และน้ำ ทำให้เป็นกรดด้วย 0.4% phosphoric acid และมี 10 มิลลิโมล tetraethylammonium bromide ผสมอยู่ โดยให้มีสัดส่วนของ acetonitrile อยู่ระหว่าง 15-20% ใช้อัตราเร็วของการไหล 0.5 ลูกบาศก์เซนติเมตรต่อนาที ปริมาตรที่ฉีดคือ 10 ไมโครลิตร และตรวจวัดที่ความยาวคลื่น 260 นาโนเมตร วิธีนี้เหมาะสมในการวิเคราะห์ตัวยาออกจากสารปนเปื้อนที่ได้จากการสลายตัวของยา cefoxitin cefazolin cefotoxime ceftazidime และ cephalexin วิธีได้ถูกประเมินความถูกต้องและเที่ยงตรงสำหรับใช้ในการวิเคราะห์หาปริมาณจากการศึกษาเน้นหาระดับการปนเปื้อนจากสารสลายตัวตัวอย่างยาฉีด cefoxitin cefazolin cefotaxime และ ceftazidime ประเมินคุณภาพจากร้อยละความบริสุทธิ์ของพีคในโครมาโทแกรม พบว่าตัวอย่างยาต้นแบบซึ่งมีราคาแพง และยาทำเลียนแบบไม่มีความแตกต่างกัน ผลิตภัณฑ์ที่มีราคาสูงไม่สัมพันธ์กับความบริสุทธิ์ของตัวยา cephalexin เป็นยาแคปซูลที่มีการทำเลียนแบบกันมากในตลาดยา ความสม่ำเสมอของปริมาณตัวยาในแต่ละหน่วย และทดสอบการละลายของตัวยาเป็นหัวข้อที่เลือกมาเพื่อศึกษาคุณภาพของผลิตภัณฑ์ยา พบว่าทุกตัวอย่างผ่านมาตรฐานตามเกณฑ์การยอมรับของเภสัชตำรับสหรัฐอเมริกา (USP 24) ราคาไม่สามารถใช้เป็นตัวบ่งชี้คุณภาพของยา cephalexin การศึกษาชีวสมมูลเป็นวิธีการประเมินคุณภาพของผลิตภัณฑ์ยาได้ดีกว่าการทดสอบในงานวิจัยนี้ แต่ทว่าไม่ใช่ขอบเขตงานวิจัยในครั้งนี้

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Abstract

A suitable mobile phase for the column of C_{18} Hypersil 5 μm 250x3.9 mm in this study is the mixture of acetonitrile and water containing 0.4% phosphoric acid and 10 mM tetraethylammonium bromide. The proportion of acetronitril was varied from 15-20%. The column was operated at a flow rate of 0.5 cm^3/min. The injection volume was 10 μl. Detection was at a wavelength of 260 nm. The method proved to adequately separate the degradation compounds from the parent compounds of cefoxitin, cefazolin, cefotaxime, ceftazidime and cephalexin. The method has also been validated for quantitative analysis. Samples of cefoxitin, cefazolin, cefotaxime and ceftazidime in intravenous form have been investigated with emphasis on the level of degradation products. The percent of peak purity from chromatograms was used to justify the quality of the products. There was found to be no difference between the generic products and the original products. The higher price of the products doesn't relate to the purity of the products in this study. There are many generic products of cephalexin capsules on the market. Content uniformity and dissolution testing have been chosen to investigate the quality of the products. It was found that every sample met the standard of The United States Pharmacopeia 24 and National Formulary 19 (USP 24). The price is also unable to indicate the quality of cephalexin capsules. However, a bioequivalence study of the products, which was out of the scope of this study, may be a better way of evaluate the quality of the products.

คำสำคัญ: cephalosporins คุณภาพ ยาเลียนแบบ
Keywords: cephalosporins, quality, generic drugs

Introduction

The cephalosporins as shown in Figure 1, semisynthetic antibiotics, are widely used for the treatment of Gram-positive and Gram-negative bacteria. They were originalyl derived from cephalosporin C, the fermented product of the mould, *Cephalosporium acremonium.* Its active nucleus, 7-aminocephalosporanic acid, is very closely related to the penicillin nucleus, 6-aminopenicillanic acid. It consists of a beta-lactam ring fused with a 6-membered dihydrothiazine ring and having an acetoxymethyl group at position 3. Cephalosporin C has a side-chain at position 7 derived from D-α-aminoadipic acid. Chemical modification of positions 3 and 7 has resulted in a series of drugs with different characteristics. Substitution at the 7-amino group tends to affect antibacterial action whereas at positions 3, it may have more of an effect on pharmacokinetic properties (Parfitt, 1999). Cephalosporins have a high share of the drug market in Thailand. There are many generic drugs with significantly lower price than the original standard drugs. The quality of the products is always in doubt, particularly for generic drugs. Quality control of cephalosporins in intravenous injection dosage form is vital to assure the amount contained in the products and the related impurities are within the correct limits. The impurities may arise in either the isolation processes of semisynthesis or decomposition products due to expiration date or the storage conditions.

Quality of cephalosporins in capsule dosage form can be indicated by the content uniformity and the dissolution testing of the products. Poor quality of the products could lead to therapeutic failure and drug-resistant organisms (Taylor et al., 2001). The method used for quality control has to be proved for adequate sensitivity and selectiveness particularly for detecting related impurities.

The purpose of this work was to determine an appropriate analytical method using HPLC for application to quality control of the series of cephalosporins in pharmaceutical dosage forms. The method should be able to reduce steps of analysis, time and resources consumed. The method was applied for analysis of the samples of cephalosporins.

Research Methodology

Material and equipment

Chromatographic separations were performed using a HPLC with diode array detector model HP 1100, operated with the Chemstation software® and incorporating Peak Purity software® (Hewlett Packard). The column was a Hypersil ODS 5 μm, 250 x 3.9 mm i.d. incorporating a Hypersil ODS 5 μm, 4 x 4 mm i.d. (Agilent Technologies) guard column.

Acetonitrile, HiPerSolv for HPLC and Tetraethylammonium bromide (TEAB), GPR®, were obtained from BDH (Poole, England). Phosphoric acid, AnalaR grade was obtained from Merck (Darmstadt, Germany). Water was purified using a Milli – Q Plus system (Millipore Co.) and all other reagents were of AnalaR or equivalent grade. Selected cephalosporins (cefoxitin, cefazolin, cefotaxime, ceftazidime and cephalexin) of standard drugs and of generic drugs were purchased from drug stores located near every university's hospital in Bangkok, the drug stores in the inner city area of Khon Kaen province and also from Srinagarind Hospital, Khon Kaen University, Khon Kaen.

Analytical method development

Chromatographic conditions: mobile phase used was a mixture of acetonitrile and water containing 0.4% phosphoric acid and 10 μM tetraethylammonium bromide. The proportion of acetronitrile was varied from 15-20%. The column was operated at a flow rate of 0.5 cm^3/min. Injection volume was 10 ml. UV detection was at a wavelength of 260 nm.

The decomposition products were simulated by treating the compounds to hydrolysis with water at room temperature for 18 hours and heat to boil for 5 and 10 minutes. The chromatograms obtained from freshly prepared solutions and treated solutions were compared for resolution of the compounds, Peak Purity software® was used to assure adequate separation of the compounds.

The method has been validated for accuracy and precision within day and between days and then was applied for determination of the quality of the samples. All intravenous (IV) injection samples were investigated for the purity of the compounds whereas the samples in capsule dosage form were analyzed under the aspects of content uniformity and dissolution testing, following the method and the criteria of The United States Pharmacopeia 24 and National Formulary 19 (The United States Pharmacopeial Convention, 2000).

Sampling method

Five cephalosporins: cefoxitin, cefazolin, cefotaxime, ceftazidime and cephalexin have been selected in this study by the concept that there are

many generic products in the drug market in Thailand. The sampling method was by buying every batch of the selected cephalosporins that were in the drug stores located near every university's hospital in Bangkok, the drug stores in the inner city area of Khon Kaen province and also from Srinagarind Hospital, Khon Kaen University, Khon Kaen.

Results and Discussion

It was found that 20% of acetonitrile in water containing 0.4% phosphoric acid and 10 mM tetraethylammonium bromide was a suitable mobile phase to separate cefoxitin, cefazolin, cefotaxime and cephalexin from their degraded products as shown in Figure 2-5. However, this mobile phase is inadequate to separate ceftazidime from its interferences. Because of the retention time of ceftazidime close to the solvent front, the percentage of acetonitrile used has to be reduced to 15% to achieve the optimum separation as the chromatogram shows in Figure 6. Purity of the parent peaks was proved by using Peak Purity software®.

Validation of the method

It can be seen that in all cases, the accuracy expressed as a percentage of spiked concentration found is between 98-102% and the coefficient of variation (%CV) is less than 1.5% as shown in Table 1. The precision of the proposed assay method within day and between day is shown in Table 2. The result of the precision within day is expressed as the coefficient of variation (%CV) and is less than 2% in all compounds. The precision between day is also shown as the coefficient of variation (%CV) and has a maximum value of 4% for over a period of three days. The correlation coefficient (r^2) of the calibration lines (n=6) of all cephalosporins were between 0.999 and 1.000 and the coefficient of variation (%CV) was less than 0.12 as shown in Table 3. All these results indicate the reliability of the method.

The related impurities of the products

The purity of the samples was evaluated by the percentage of the parent peak out of the total peaks in the chromatograms. An example of the result using the Peak Purity software® of cefoxitin after boiling for 10 minutes is shown in Figure 7. The high purity of the peak of cefoxitin in the chromatogram indicates the selectivity of the method. The peak of cefoxitin had no interference from the degradation products obtained from simulated stress conditions.

Quality of the IV injection samples; cefoxitin, cefazolin, cefotaxime and ceftazidime

Purity and the price of the IV injection samples of cephalosporins are shown in Table 4. It may be concluded that the price has no correlation to the quality in the aspect of the purity of the marketed products of cephalosporins; cefoxitin, cefazolin, cefotaxime and ceftazidime. However, other quality aspects of cephalosporins IV injection samples i.e. sterility test, assay etc, were out of the scope of this study.

Quality of cephalexin capsule samples

The content uniformity of all cephalexin samples were within the criteria of USP 24. For all individual cephalexin capsules, the labeled percentage amount lay between 100.73 and 111.40 which were within the range of 85.0% to 115.0% of the label claim and the relative standard deviation from ten capsules of each cephalexin batch was less than 6.0% as shown in Table 5.

The results of dissolution testing using apparatus 1 (100 rpm) and water as medium are shown in Table 6. The amount dissolved of all cephalexin capsules samples was greater then 80% of the label claim (%Q) within 30 minutes which passed the dissolution test standard of USP 24.

The quality of each batch of cephalexin capsule represented as the results of %CV of the content uniformity and the dissolution testing compared to the price are shown in Table 7. It is seen again that we cannot justify the quality of cephalexin products solely by the price.

Conclusion

It may be concluded that the price of the Thai marketed cephalosporins products cefoxitin, cefazolin, cefotaxime, ceftazidime and cephalexin may not correlate to the quality of the products in this study. The high price of the products produced by international companies may not mean higher quality than the cheap products produced locally. Interchangeability of the products should be considered. However, the meaning of quality has a much greater extent than the scope specified in this study. Further work needs to be done to assure quality of the products.

Acknowledgement

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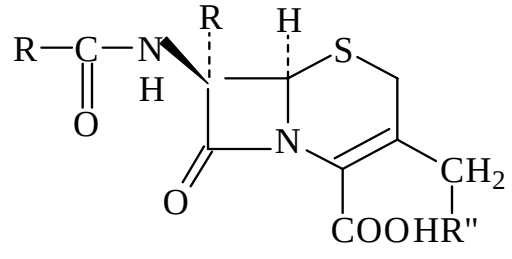

Figure 1. Structure of cephalosphorins.

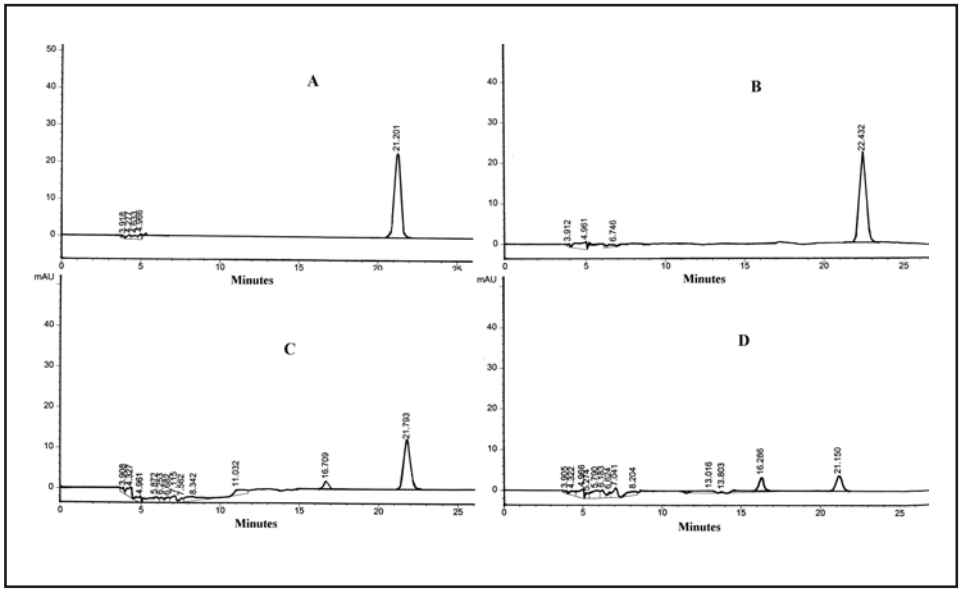

Figure 2. Chromatograms of cefoxitin in water freshly prepared (A), stored 18 hours at room temperature (B), boiled for 5 minutes (C) and boiled for 10 minutes (D).

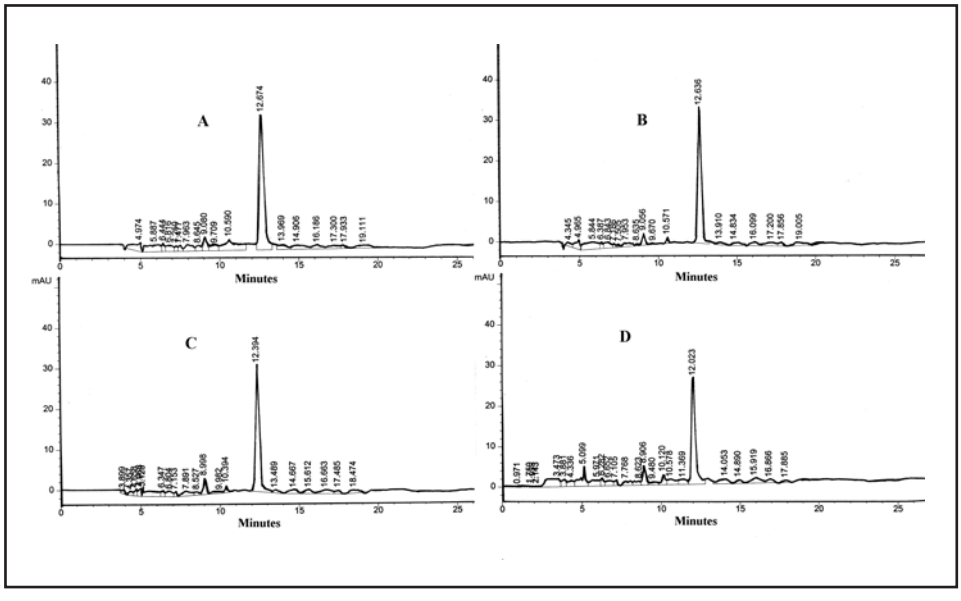

Figure 3. Chromatograms of cefazolin in water freshly prepared (A), stored 18 hours at room temperature (B), boiled for 5 minutes (C) and boiled for 10 minutes (D).

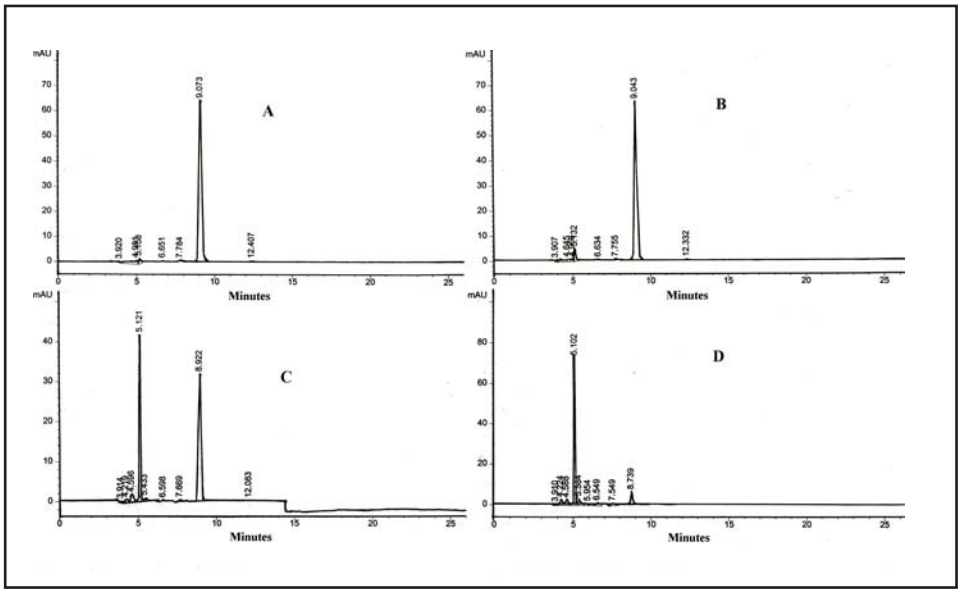

Figure 4. Chromatograms of cefotaxime in water freshly prepared (A), stored 18 hours at room temperature (B), boiled for 5 minutes (C) and boiled for 10 minutes (D).

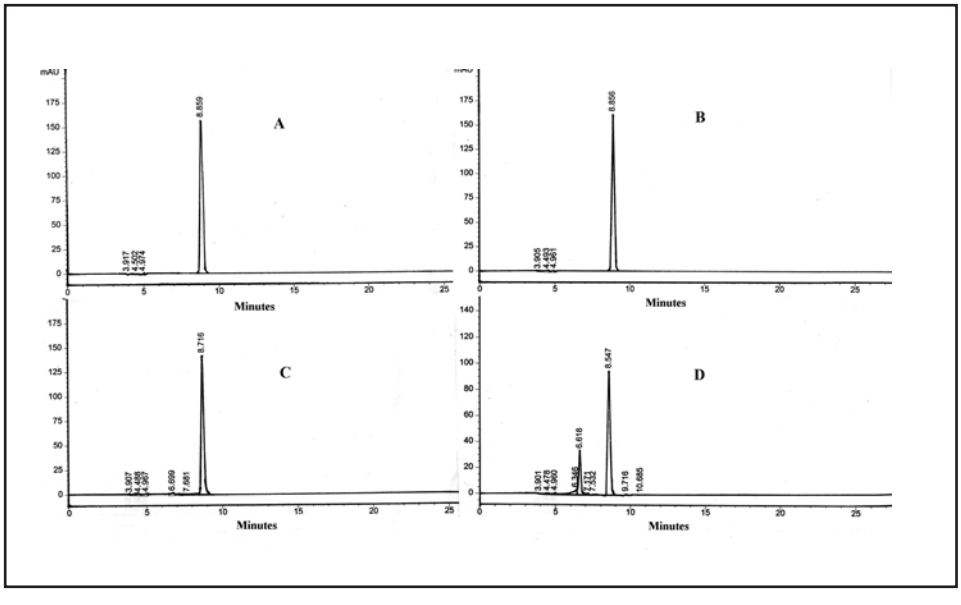

Figure 5. Chromatograms of cephalexin in water freshly prepared (A), stored 18 hours at room temperature (B), boiled for 5 minutes (C) and boiled for 10 minutes (D).

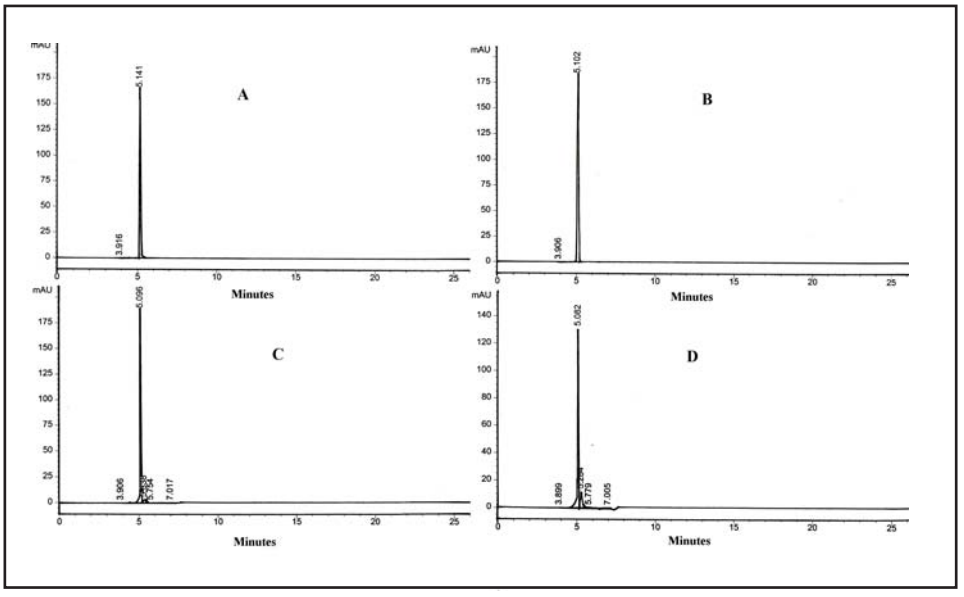

Figure 6. Chromatograms of ceftazidime in water freshly prepared (A), stored 18 hours at room temperature (B), boiled for 5 minutes (C) and boiled for 10 minutes (D).

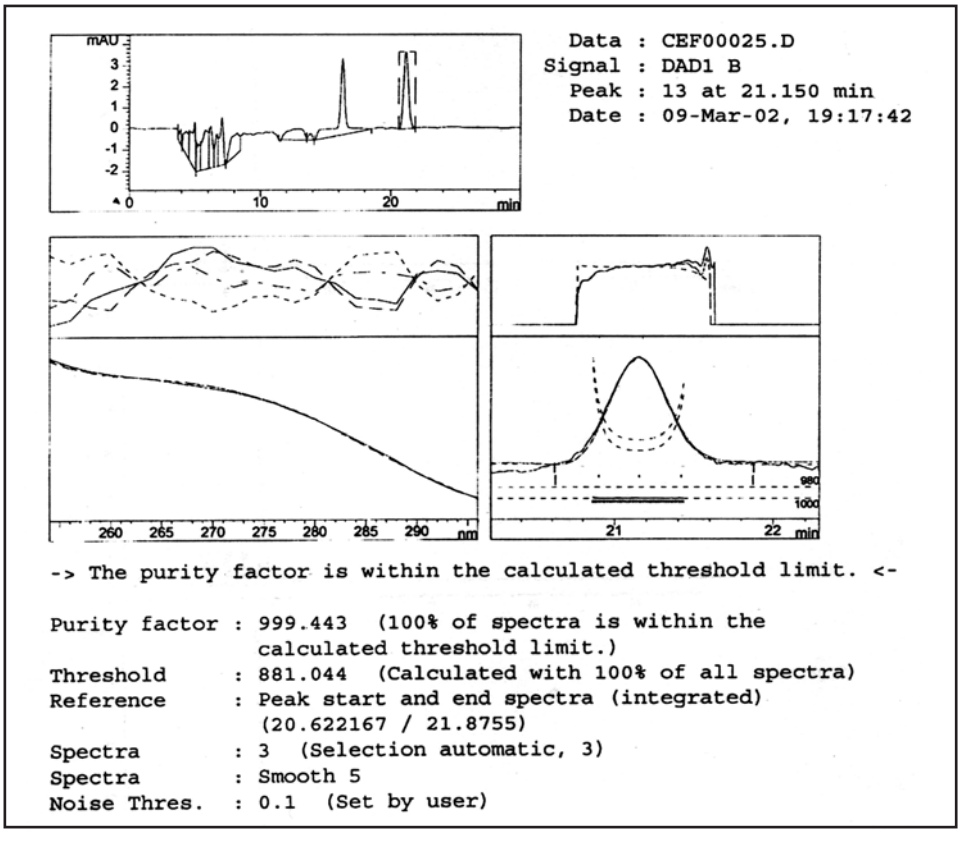

Figure 7. Purity of the peak of cefoxitin in the chromatogram, after boiling for 10 minutes in water, measured using the Peak Purity software®. The peak of cefoxitin was not affected by the degradation products

Table 1. Showing the accuracy of the analytical method (n=10).

Compound	True value (μg/ml)	Recovered value (μg/ml)	%found	%CV
Cefoxitin	69.18	68.45	98.95 ± 0.82	0.83
Cefazolin	68.46	69.59	101.65 ± 1.35	1.33
Cefotaxime	74.16	73.99	99.78 ± 0.33	0.34
Cephalexin	72.54	71.76	98.92 ± 0.18	0.18
Ceftazidime	67.62	67.50	99.83 ± 0.14	0.14

Table 2. The precision within day and between day of the analytical method for the assay of cephalosporins.

Compound		Measured value (peak area/μg/ml)	
		Mean ± SD	%CV
Within day (n=10)	Cefoxitin	14.79 ± 0.12	0.84
	Cefazolin	6.24 ± 0.08	1.33
	Cefotaxime	38.83 ± 0.13	0.34
	Cephalexin	17.70 ± 0.03	0.19
	Ceftazidime	31.96 ± 0.05	0.15
Between day (n=22)	Cefoxitin	14.81 ± 0.07	0.48
	Cefazolin	3.85 ± 0.12	3.22
	Cefotaxime	37.57 ± 1.42	3.78
	Cephalexin	17.73 ± 0.09	0.52
	Ceftazidime	32.16 ± 0.30	0.92

Table 3. The constancy of linearity of cephalosporins (n=6) over a period of six days.

Compound	Slope		Correlation coefficient (R^2)	
	Mean ± SD	%CV	Mean ± SD	%CV
Cefoxitin (n=6)	15.72 ± 0.85	5.38	0.999 ± 0.00	0.07
Cefazolin (n=5)	5.08 ± 0.89	17.50	1.000 ± 0.00	0.04
Cefotaxime (n=6)	37.75 ± 1.52	4.03	0.999 ± 0.00	0.07
Cephalexin (n=6)	18.38 ± 0.96	5.24	0.999 ± 0.00	0.12
Ceftazidime (n=3)	33.75 ± 0.02	0.07	1.000 ± 0.00	0.00

Table 4. Comparison of the price with the results of the purity measurements of the cephalosporins IV injection samples.

Compound	Sample No.	Price (Baht per unit)	Detected interfering peaks (%)	Chromatographic purity (%)
Cefoxitin	1	150	0.12	99.88
	2	185	0.10	99.90
Cefazolin	1	60	0.17	99.83
	2	45	0.10	99.91
	3	50	0.12	99.88
	4	80	0.72	99.28
	5	60	0.15	99.85
Cefotaxime	1	250	0.76	99.24
	2	80	0.66	99.34
	3	130	0.33	99.67
	4	51	0.27	99.73
Ceftazidime	1	100	ND	100.00
	2	400	ND	100.00
	3	80	ND	100.00

Table 5. Results of the content uniformity measurement of eleven cephalexin samples.

Sample no.	% labeled amount	
	Mean ± SD	%CV
1	104.89 ± 5.96	5.68
2	100.73 ± 5.95	5.90
3	103.66 ± 2.85	2.75
4	111.40 ± 2.64	2.37
5	103.66 ± 2.85	2.75
6	103.66 ± 2.85	2.65
7	106.14 ± 3.03	2.85
8	110.63 ± 1.58	1.43
9	109.75 ± 4.02	3.67
10	106.06 ± 4.00	3.77
11	108.21 ± 2.64	2.44

Table 6. Results of dissolution test of eleven cephalexin samples.

Sample no.	% labeled amount			
	Mean ± SD	%CV	Maximum	Minimum
1	105.39 ± 3.38	3.21	111.88	102.56
2	106.66 ± 2.97	2.78	109.40	101.48
3	106.69 ± 2.65	2.48	109.41	101.78
4	106.69 ± 4.38	4.10	111.34	99.85
5	105.65 ± 1.28	1.21	107.72	104.02
6	105.45 ± 4.29	4.07	108.53	98.43
7	107.07 ± 5.51	5.15	113.49	99.64
8	111.92 ± 1.69	1.51	114.20	109.53
9	103.26 ± 2.01	1.95	106.48	101.66
10	102.27 ± 5.26	5.14	105.67	91.69
11	102.93 ± 1.64	1.59	104.87	101.18

Table 7. Comparison of the price with the results of the %CV obtained from the content uniformity and the dissolution testing of the cephalexin samples.

Sample no.	Dose per capsule (mg)	Price (Baht per unit)	Content uniformity (%CV)	Dissolution test (%CV)
1	500	5	5.68	3.21
2	500	5	5.90	2.78
3	250	3	2.75	2.48
4	500	6	2.37	4.10
5	500	6	2.75	1.21
6	500	5	2.65	4.07
7	500	17	2.85	5.15
8	500	45	1.43	1.51
9	250	3.5	3.67	1.95
10	250	3	3.77	5.14
11	250	5	2.44	1.59