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藥物食品分析
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應用HPLC方法於Cefadroxil製劑之品質管制

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摘　要

在七十九年度發展出抗生素Cefadroxil之高效液相層析法，此法可用於鑑別及測定Cefadroxil原料藥及膠囊製劑之力價，簡單、迅捷且具特異性，可用於取代微生物法來測定Cefadroxil之力價。本實驗乃應用此HPLC方法針對市售之五家不同廠牌Cefadroxil膠囊製劑品質管制之研究，包括容量均一度(Content uniformity)，模擬處方試驗之回收率(Recovery)及溶離度試驗(Dissolution test)等。結果顯現不同廠牌製劑之HPLC圖譜，其滯留時間和回收率不會受到製劑賦形劑之影響，顯示此法及分析條件確可應用於藥廠之品質管制。

前　言

目前有許多文獻利用HPLC法分析抗生素[1-5]及分離Penicillin與Cephalosporin類化合物[6-9]；亦有以HPLC法取代微生物法[10,11]及以HPLC法測定生物體液中Cefadroxil[12-14]和藥效動力學[15,16]之報導。

目前公定書中Code of Federal Regulation[17]是以羥胺呈色法或微生物法(Microbiological agar diffusion)來分析Cefadroxil原料藥及其製劑，日本抗生物質基準解說[18]是以微生物法來測定Cefadroxil之力價，然各法中仍以微生物法所得之結果爲主。一般而言，抗生素鑑別用的呈色法[19,20]或薄層層析法(T.L.C)[21]，力價測定用的光學法(羥胺呈色法或酸解法)或微生物法，均不具特異性；又傳統微生物分析法所得之結果差異性大且費時，因此於七十九年度研究出一種值得信賴、準確、省時又簡單的HPLC方法[22]來分析Cefadroxil原料藥及膠囊製劑之檢體。

利用前所發展之HPLC法[23]針對市售五家藥廠所生產之Cefadroxil膠囊製劑進行品質管制研究，以評估此方法運用於品質管制之可行性。本研究包括藥廠一般例行檢驗項目如溶離度試驗及容量均一度等，而對不同廠別所添加不同賦形劑對分析圖譜及定量所造成之影響亦可做一探討。

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材料與方法

一、試藥與試劑：

Acetonitrile(J.T. Baker INC. HPLC級)，磷酸氫二鉀及磷酸二氫鉀(和光，試藥級)，Cefadroxil標準品(藥物食品檢驗局製，力價934.8 μg/mg)，內部對照標準品Dimethylphthalate(Merck Schuchardt)，榮富多信膠囊(250 mg，聯邦製藥，批號CF, CO2J31)，KDM-CD膠囊(500 mg，景德製藥)，多福黴素膠囊(500 mg，永信藥品，批號CIC 13009)，賜福力膠囊(500 mg，中國化學製藥，批號KBT049)，賜華力膠囊(500 mg，生達製藥，批號CC03016)。

二、HPLC法：

㈠內部對照標準品(Internal standard)溶液之配製：

精確量取適當量之Dimthylphthalate，以acetonitrile:H_2O＝1：1之溶液稀釋成0.03 mg/ml之貯備溶液。

㈡標準品溶液之配製：

精確稱取適當量之Cefadroxil對照標準品，並加入內部對照標準品之貯備溶液，再以0.1 ml磷酸鹽緩衝溶液(pH4.5)溶解並稀釋成每ml含0.1 mg Cefadroxil(力價)及0.3 μg dimethylphthalate之標準品溶液。

㈢儀器及分析條件：

1.儀器：Gasukuro KOGYO ING Liquid Chromatography

2.逆相層析管：μ-Bondapak C_{18}, 300 mm × 3.9 mm I.D.,Waters P/N 27324

3.移動相：以Acetonitrile：0.01M磷酸鹽緩衝液＝60：40比配製成移動相，並以0.45 μm(Acrodisc^R,LCPVDF)溶媒濾清器過濾

4.UV偵測器：Gasukuro spectro Detector 502U UV 254nm

5.記錄器：Gasukuro Chromatocorder 12

6.注射量：20 μl

㈣檢體溶液配製：

1.市售檢體添加Cefadroxil之溶液配製：

於市售膠囊檢體中，研磨成均均之粉末，分取四份，每份各精取25 mg(力價)，分別加入0 mg, 5 mg, 7.5 mg, 10 mg, (potency)之Cefaadroxil標準品(即約實際檢體取量之0%、20%、30%、40%)之適當之0.1 m PBS (pH4.5)稀釋，使成含有di-

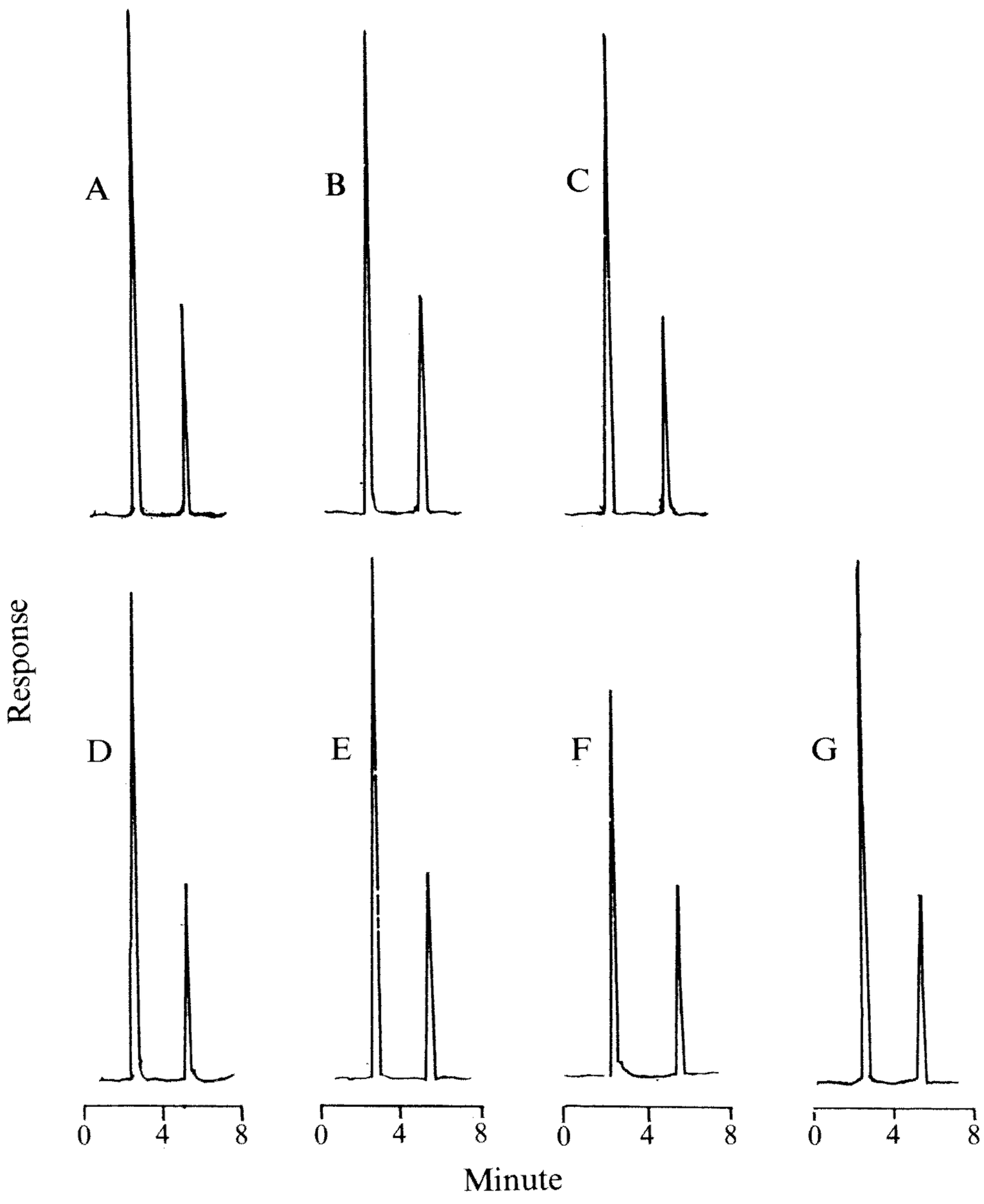

Figure 1. Typical chromatograms of cefadroxil preparation:
(A)house standard; (B)bulk drug subatance; (C)Brand A capsule; (D)Brand B capsule; (E)Brand C capsule; (F)Brand D capusle; (G)Brand E capsule;
peaks: 1, cefadroxil; 2, dimethylphthalate

methylphthalate 0.3 μg/ml及Cefadroxil各爲0.25 mg/ml, 0.3 mg/ml, 0.325 mg/ml, 0.35 mg/ml之溶液。做回收率試驗時，再由各個溶液取20 μl注入HPLC。

2.容量均一度試驗之溶液配製：

於市售檢體中，各取不同廠牌之膠囊10粒，精確稱取其各別之內容量並研麼成均勻粉末，精確秤取相當於Cefadroxil(力價)之檢體，加入適量之內部對照標準品溶液，並以適量之0.1 M PBS(pH4.5)成每ml含0.25 mg Cefadroxil(力價)及0.3 μg dimethylphthalate之檢體溶液。

3.溶離度試驗之溶液配製：

依據U.S.P XXI[10]版所載，以蒸餾水900 ml爲溶離度試驗液，溫度設於37±0.5°C，試驗籃轉速爲100 r.p.m。試驗時每槽放置一顆膠囊，每隔5、10、15、20、30、45、60分鐘各由槽中取出10 ml溶液，並以蒸餾水10 ml補足槽中體積。將上述取出之溶液加內部對照標準品溶液1 ml，過濾後取20 μl打入HPLC。本試驗之對照溶液配製法爲取6顆與檢體相同廠牌之膠囊，取出內容物精確秤重並混合，精取相當於一顆平均重量之粉末溶於蒸餾水900 ml中，使其充份溶解後取10 ml並加1 ml內部對照標準品溶液，過濾後同樣打20 μl入HPLC。

結果與討論

圖一爲各類Cefadroxil製劑之層析圖。以dimethylphthalate爲內部對照標準品，針對Cefadroxil之各類製劑進行分析，包括：局製備標準品、原料藥、及五家不同廠牌之膠囊製劑。

Table 1. Content uniformity of Cefadroxil in different brands, expressed as % of label Claim.

Capsules No.	A	B	C	D	E
1	96.9	98.9	103.3	102.5	103.7
2	102.8	100.6	97.2	101.4	106.6
3	102.9	95.4	97.0	103.0	105.9
4	105.0	98.5	95.3	102.0	102.6
5	104.3	96.3	97.4	103.0	101.9
6	101.7	98.5	104.4	101.9	99.6
7	105.0	96.3	99.9	102.8	107.6
8	101.0	97.9	99.4	101.0	101.0
9	100.4	98.1	100.6	99.5	104.0
10	104.0	97.2	102.7	104.1	100.7

Diemthylphthalate滯留時間約爲5分鐘，Cefadrosil之滯留時間則均在2分鐘以內。顯示本法可適於市售不同廠牌Cefadroxil膠囊製劑之分析，其滯留時間並不因各廠所加賦形劑的不同而受到影響。

應用此HPLC法於五家廠牌Cefadroxil膠囊之內容量測定，其結果示於表一，以HPLC法取代微生物法，檢驗時間、人力物力均可節省很多，對於藥廠每天例行繁重的品管工作有極大助益。表二則爲市售膠囊檢體中分別加入約標示力價含量20%，30%，及40%之Cefadroxil標準品以測定不同廠牌添加不同賦形劑對平均回收率的影響。其回收率分爲A廠102%，B廠99.83%，C廠98.69%，D廠99.26%，E廠98.9%，由此可知，在此HPLC方法操作下，所得之回收率並不會因賦形劑之不同而受影響。

各家廠牌Cefadroxil膠囊製劑之溶離度曲線圖於圖二，顯示各家廠牌製劑雖均在藥典所規定之45分鐘內溶離，但其速率卻因添加不同賦形劑而不同，同時溶離之程度也會受到賦形劑的影響，這些曲線亦可做爲藥廠品質管制及處方開發研究之參考。

結　論

以微生物法檢驗，至少需16-18小時，且特異性不高，操作人員亦需有純熟之技術才能將差異性降至最低；而本HPLC法只要在6分鐘內便可同時得知鑑別與力價之結果，不僅操作容易，特異性、再現性都高。除此之外，對市售五家不同廠牌膠囊製劑作品質管制，發現無論添加何種賦形劑，本條件均可適用。故考量實驗精確性、時間及人力，本HPLC

Table 2. Recovery of Cefadroxil from spiked samples.

Amount added (mg) / Brand	20	30	40	Average
	% of Recovery *			
A	101.9	104.4	99.8	102.0
B	100.6	97.2	101.7	99.8
C	98.1	98.3	99.6	98.7
D	97.0	99.0	101.8	99.3
E	100.1	98.0	98.7	98.9

*an average of 6 determinations

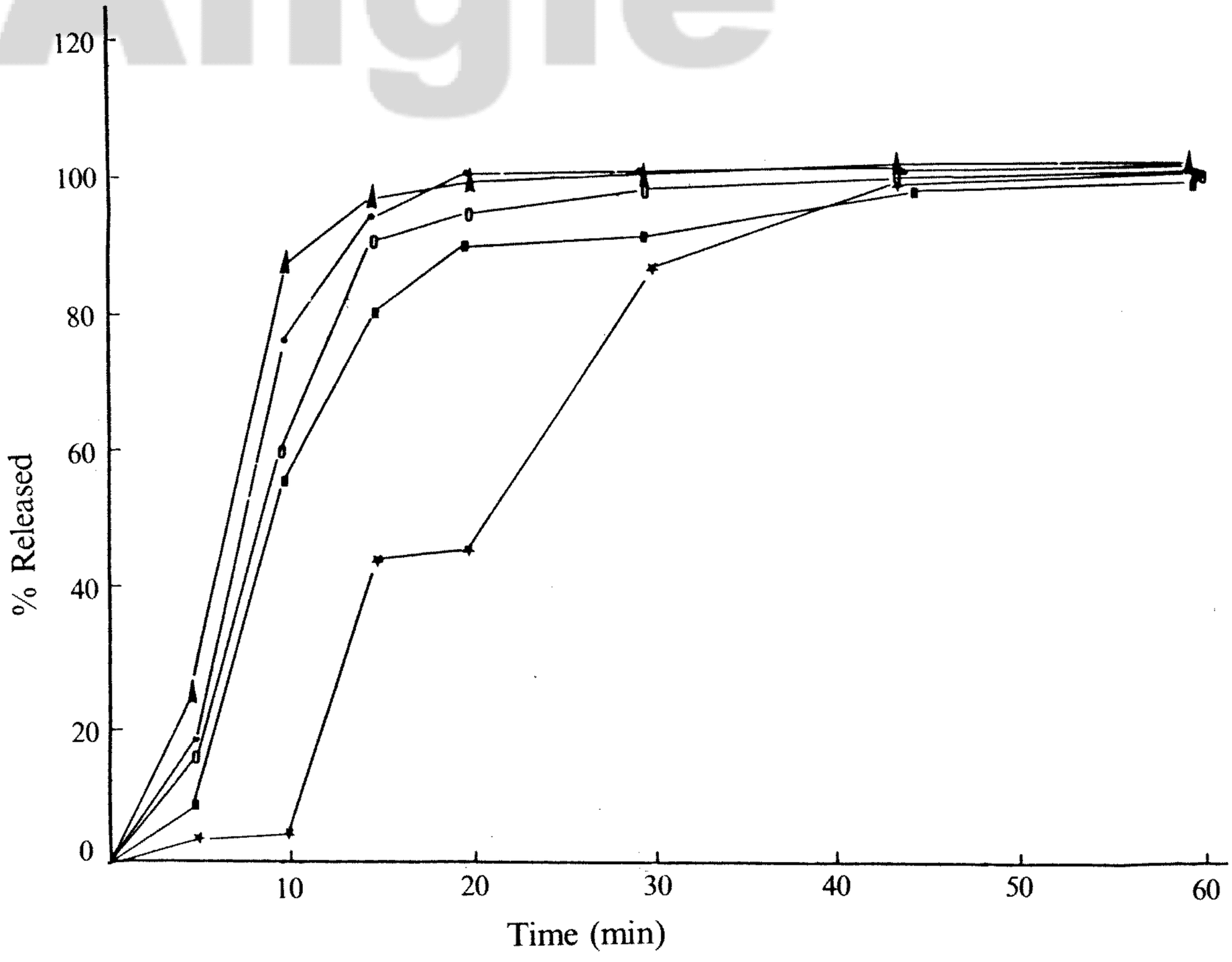

Figure 2. Dissolution rates of cefadroxil in water at 37°C
Brand A(0), Brand B(■), Brand C(*) Brand D(·) and Brand E(▲).

法確可提供予國內各藥廠做爲Cefdroxil製劑之品質管制及研究開發時使用。

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Application of HPLC Method for Quality Control of Cefadroxil

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ABSTRACT

Our previous studv has shown that HPLC method is a suitable substitute for the microbiological method for potency assays and stability studies of cefadroxil preparations. The samples were analysed on μ-Bondapak C_{18} column with a mobile phase consisting of acetonitrile and phosphate buffer. The assay was also applied successfully to five different commercial brands and proved to be free of interference from excipients normally used in formulations. The contents of unifomity and dissolution rates in different brands were performed. The results have shown that this analytical method could be utilized readily for routine quality control of cefadroxil pharmaceuticals, since it offers a simple system and short analytical time coupled with reproducibility and accuracy.

Key words : Cefadroxil, quality, HPLC.