

THE APPLICATION OF METHANOL-WATER AS LIQUID CHROMATOGRAPHY MOBILE PHASE FOR DETERMINATION OF AMOXICILLIN-POTASIAM CLAVULANATE IN TABLET



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OUTLINE

- Background and Purposes
- Method
- Result
- Conclusion

Background

Amoxicillin-Potassium Clavulanate :

Combination drug

- How to determine each substance without any interfere from other?



Analysis Method -> volumetri, elektrometri,
spektrofotometri

-> Chromatography : TLC,
HPLC : column, eluen

- How is the method validation?
- How is the aplication of proposed method?

PURPOSES

- To find the optimum composition of the mobile phase to give good separation
- To validate the proposed method
- To apply the proposed method for determining Amoxicillin-Potasium clavulanate in dosage form

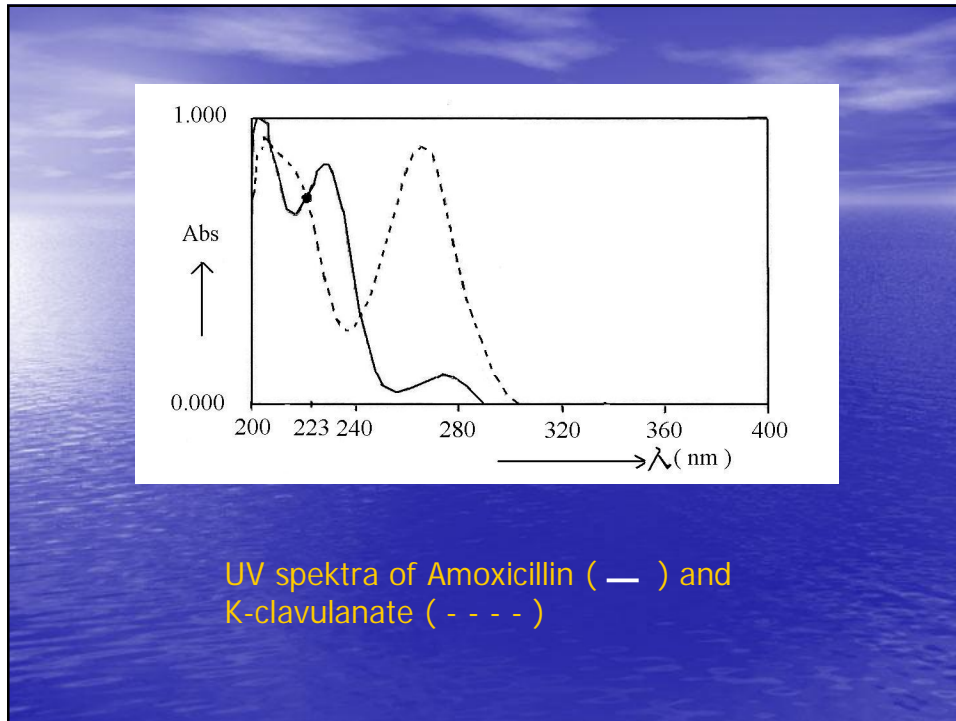
METHOD

- Optimization of mobile phase → comprise Methanol : Water in 5 composition :
75 : 25, 80 : 20, 85 : 15, 90 : 10, 95 : 5 (%v/v)
- Method Validation parameters [ICH, USP XXV] : selectivity (R_s),
linearity (r , V_{xo}),
precision (RSD),
accuracy (%R).

METHOD

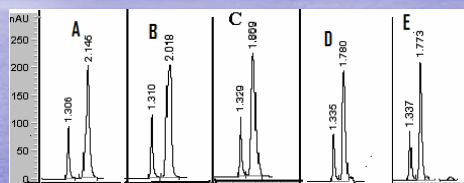
HPLC system :

- Agilent 1100 series with LC pump, injector, DAD UV detector
- Condition :
 - * Column : Lichrospher RP-C18 (4x25x5)
 - * Flowrate : 1mL/min
 - * T : 40°C
 - * Peak detection : UV at 223nm



RESULT

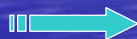
- Mobile Phase Optimization



Chromatogram of various MP composition :

- A = 75:25
- B = 80:20
- C = 85:15
- D = 90:10 (MeOH:water,
- E = 95:5 % v/v)

MP (MeOH : water)	Retention time (Rt)		α	Resolution (Rs)
	Amox	K-clav		
75 : 25	2,145	1,306	1,64	3,57
80 : 20	2,018	1,310	1,54	2,61
85 : 15	1,869	1,329	1,41	2,41
90 : 10	1,780	1,335	1,33	2,22
95 : 5	1,773	1,337	1,33	2,20

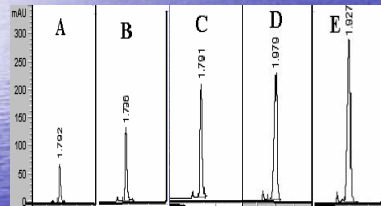


RESULT

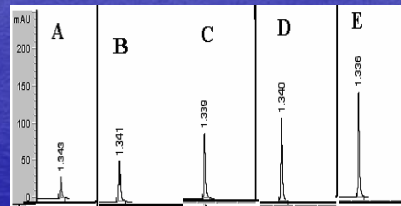
- Method Validation

Selectivity : Rs

Linearity :

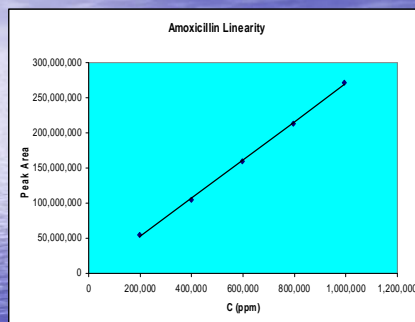


Amoxicillin : A = 19,9 ppm
 B = 39,8 ppm
 C = 59,7 ppm
 D = 79,6 ppm
 E = 99,5 ppm

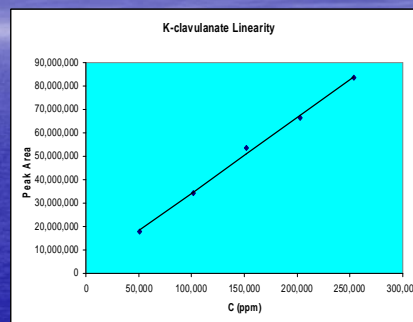


K-clavulanat : A = 5,075 ppm
 B = 10,150 ppm
 C = 15,225 ppm
 D = 20,300 ppm
 E = 25,375 ppm

RESULT



$Y = 27,1618X - 15,4563$
 $r = 0,9997$
 $V_{xo} = 1,4743\%$



$Y = 32,2628X + 20,6368$
 $r = 0,9985$
 $V_{xo} = 3,3835\%$

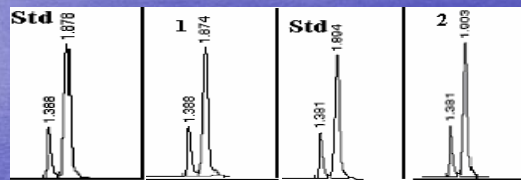
RESULT

- Accuracy (%R), Precision (KV)

Repli- ca- tion	Sample weight (mg)	Amoksisilin			K-clavulanate		
		Weigh (mg)	Recover (mg)	%R	Weigh (mg)	Recover (mg)	%R
A	111,7000	44,7025	45,2567	101,2398	11,1805	11,2182	101,2182
B	101,2000	40,5004	40,7567	100,6328	10,1295	10,4333	102,9992
C	102,2000	40,9006	40,2700	98,4582	10,2296	10,2467	100,1672
D	102,3000	40,9406	41,7900	102,0747	10,2397	10,3333	100,9141
E	100,4000	40,1802	40,4833	100,7544	10,0495	10,2267	101,7633
F	101,2000	40,2203	40,2900	100,1733	10,0595	10,2300	101,6949
				X = 100,5555%	X = 101,4595%		
				SD = 1,2138	SD = 0,9533		
				KV = 1,2071%	KV = 0,9396%		

RESULT

- Aplication



Replicate	Rt (min)		α	Rs
	Amoxicillin	K-clavulanate		
1.	1,886	1,385	1,36	2,17
2.	1,874	1,388	1,35	2,13
3.	1,903	1,381	1,38	2,22

RESULT

Amoxicillin

Rep	C Standard (ppm)	Area		Weight (mg)		% S
		Standard	Sample	Label	Recover	
1.	63,5000	2324,41821	2342,25171	500	501,5190	0,3038%
2.	63,5000	2206,34863	2276,48584	500	501,9384	0,3877%
SD = 0,2966 X = 501,7287 KV = 0,0591% Average % S tablet "A" = 0,3456%						

K-clavulanate

Replicate	C Standard (ppm)	Area		Berat (mg)		% S
		Standard	Sample	Label	Recover	
1.	15,7000	746,75330	782,18243	125	128,8931	3,1145%
2.	15,7000	706,63208	767,17542	125	130,5787	4,4630%
SD = 1,1919 X = 129,7359 KV = 0,9187% Average % S tablet "A" = 3,7888%						

CONCLUSION

- The proposed method is suitable for assay of Amoxicillin – K clavulanate combination in tablet.
- To apply this method in bioanalysis need further confirmation

The 8th Asian Conference on Clinical Pharmacy: "Toward Harmonization of Education and Practice of Asian Clinical Pharmacy"

