

*

HPLC

Validated	Selective	Sensitive
Degradation	Assay	Stability-indicating
/85	Impurities	products
210	C18	Excipients
: 3.5 = pH	UV	Detection
Forced degradation conditions		Buffer
Selectivity		.15
. / 2 0.2		.Recovery
Linearity	.%2	
Selectivity		.Accuracy
		Range

*

Stability-indicating HPLC Assay Method of Aspartame

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Abstract

The purpose of this work is to develop a sensitive, selective and validated stability-indicating HPLC assay for the analysis of aspartame tablets.

Aspartame and its potential impurities or degradation products are analyzed on C₁₈ Column using a mobile phase containing monobasic potassium phosphate buffer solution (pH = 3.5) and acetonitrile (85: 15).

The samples were detected using a UV detector at 210 nm.

The forced degradation conditions include the effect of heat, oxidation, light, and acid – base hydrolysis to get the degradation products.

The effect of many pharmaceutical excipients on the selectivity and recovery was studied.

The linear range of aspartame is between 0.2 and 2 mg/ml.

The developed method shows a separation of aspartame potential impurities and its degradation products in one run without interference from the tablet excipients. The relative standard deviation for instrumental precision is not more than (2%).

The selectivity, linearity, range, and accuracy of the developed method show acceptable value.

The developed method is suitable for quality control and stability studies of aspartame tablets.

Key Words: Aspartame, Stability-indicating, HPLC.

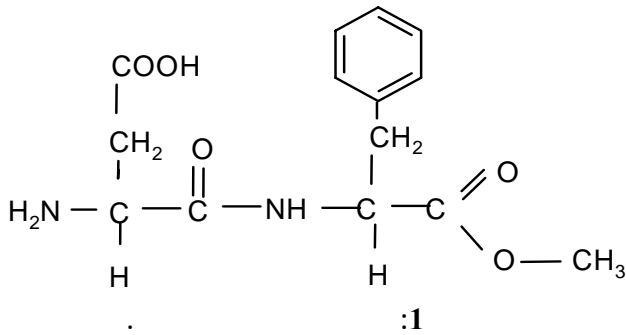
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Faculty of Pharmacy -Damascus University

Disorders **L-aspartyl-L-phenylalanine-1-methyl ester**
1 [(APM)]

Obesity

J.Schlatter 1965
Searle & CO
Nutrasweet^(R)

(1)



L - phenylalanine (as the methyl ester (Phe))
L - aspartic acid

(2) 246 - 247

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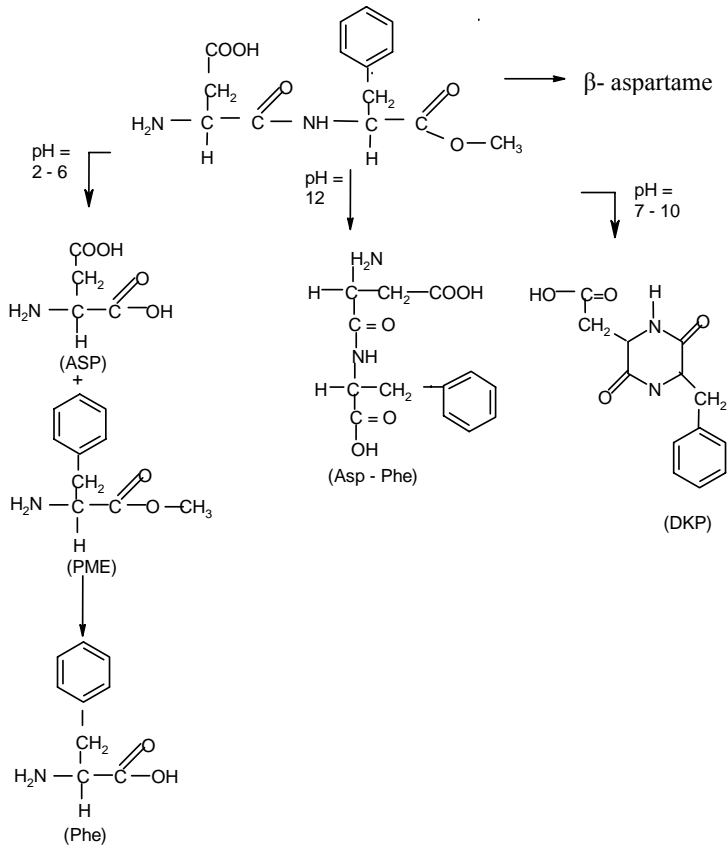
pH

(14- 3)

- L – aspartic acid (Asp)
- L – aspartyl phenylalanine (Asp – Phe)
- L – phenylalanine methyl ester (PME)
- L – phenylalanine (Phe)
- 3,6 – Dioxo – 5 – phenylmethylpiperazine acetic acid (diketopiperazine DKP)
- β - aspartame

:2 (7)

Prodolliet



Prodolliet

:2

(15)

Prankerd, RJ

(8)

Revered phase

(1)

(16 1)

(17)

(14)

(19-18)

(20)

(21)

-22 16 13 7)

(25)

(26)

(27)

(13)

Pattanaargson

250 x 4.6 mm C₁₈
 : 210 214
 Fluka Chemi, Aldrich Chemical
 CO, Sigma Chemical CO, Merck
 K GaA Chemical Reagents. /
 HPLC - .Capacity Factors
 Merck -
 -
 Reagent grade (28) pH
 -
 - pH
 Shimadzu LC - 10 AD
 Shimadzu SIL - 10 ADVP Resolutions
 . Peaks
 LC - 10
 .AD
 C TO - 10 A Column
 Oven .()
 SPD - 10 AV UV - Visible :
 () : -
 Shim - pack, SLC - ODS
 (250 x 4.6 mm) particle
 .size (5 μm)

/ 5 :
pH =
-3 15 / 85 3.5
/ 50 pH
:
-4 210
10 - 20 / 1
20 + 25
0.2) :
(
10 - GL - Sciences (250 x 4.6
20 + mm) particle size (5 µm)
0.2 :
pH =
4.3
10 - pH
%10 10 + 214 210 :
30 2 :
10 - 40
60 :
10 - + : -1
200) 1 / 9
-2

	Retention times	25	W
	Spiking	/ 5	(-
		20	()
			0.2
		20	()
			0.2
			-
System	Suitability	Parameters	4 100
1	25	/	
			30
	/ 0.2		
Precision	/ 2		
			40
	/ 25		
		214	210
		Method	Validation
Accuracy			
Recovery			

Precision of the

method :

% 35

- % 15

% 25

%5

%50

%150

%100

Robustness

10

/ 0.1

0.45 Millipore

.HPLC

Linearity of calibration curve

Correlation

factor

Combined uncertainty

Combined uncertainty = : Instrumental

$$\sqrt{(SD_1)^2 + (SD_2)^2 + (SD_3)^2 + \dots}$$

Precision

:

1

Reagents

System

suitability

requirements

Relative

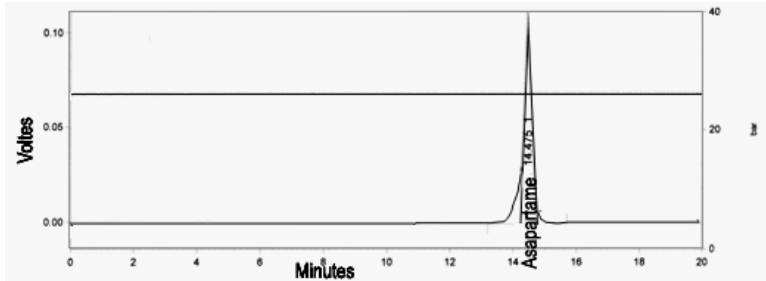
Standard

Deviation

.Means

% 0.83 Standard solution

.1 Relative Standard Deviation



:1

.System sensibility test

:1

SST	16/06/2004			
S.N.	Reading	Th.P. (N)	T.F.	R
Std - 1	2151296	12789.47	0.8	0
Std - 2	2115904	1293306	0.79	0
Std - 3	2123417	12961.38	0.8	0
Std - 4	2111793	13130.13	0.8	0
Std - 5	2106904	13172.69	0.8	0
Avg	2121862.8		Avg	0.000
SD	17531.4			
RSD	0.83			

(R² = 0.998)

2

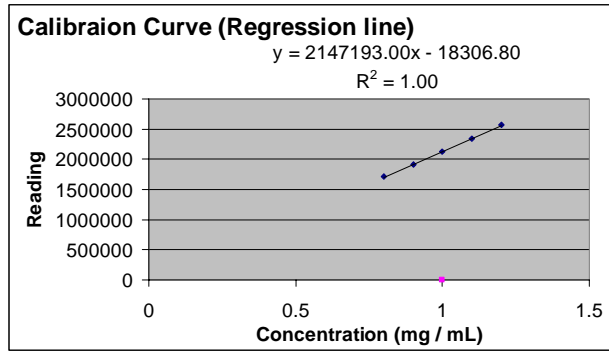
2

Correlation factor

:2

1 Linear & Working range

S.N.	Conc(%)	Conc (ug/mL)	Reading	Predicted Reading	KF	R	R(%)
1	80	0.8	1707178	1699447.6	2133972.50	7730.40	0.45
2	90	0.9	1909999	1914166.9	2122221.11	-4167.90	-0.22
3	100	1	2121471	2128886.2	2121471.00	-7415.20	-0.35
4	110	1.1	2340018	2343605.5	2127289.09	-3587.50	-0.15
5	120	1.2	2565765	2558324.8	2138137.5	7440.20	0.29
r	0.9998						
Slope	2147193.0						
Intercept	-18306.80						

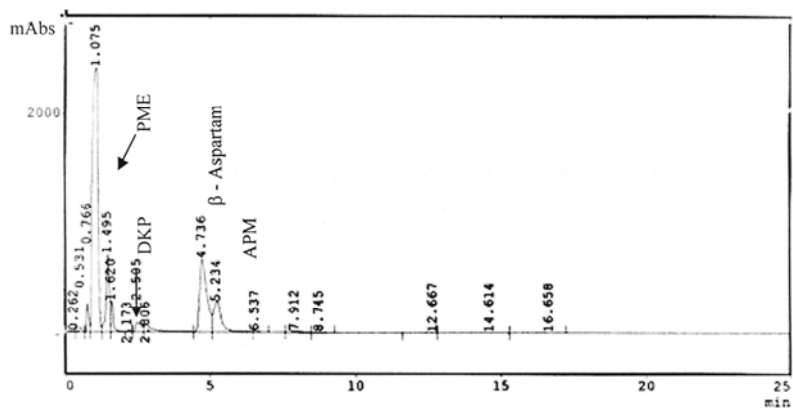


:2

(15)25

3

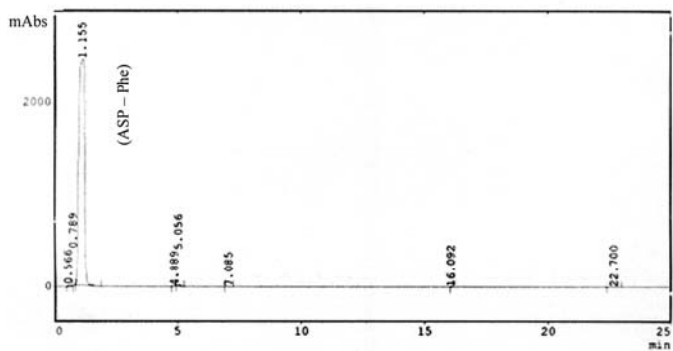
L -
 phenylalanine methyl ester (PME)
 -β



USP₂₅

:3

4



USP₂₅

:4

Chromatographic

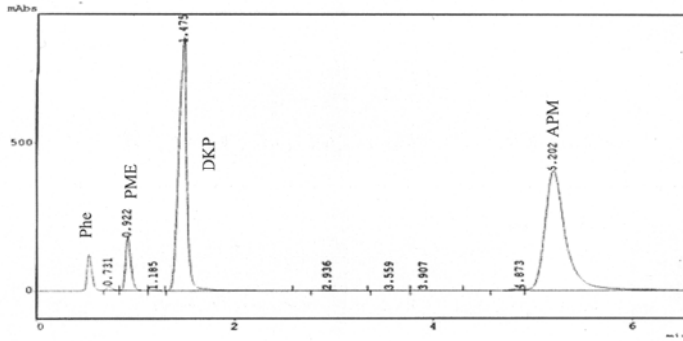
6 purity DKP

5

- β

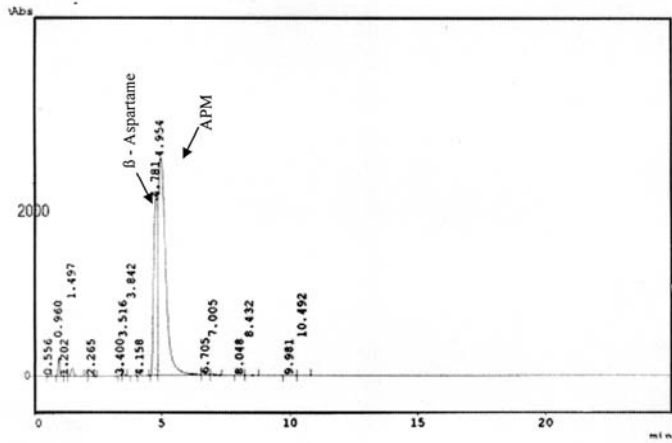
β

- β



USP₂₅

:5



USP₂₅

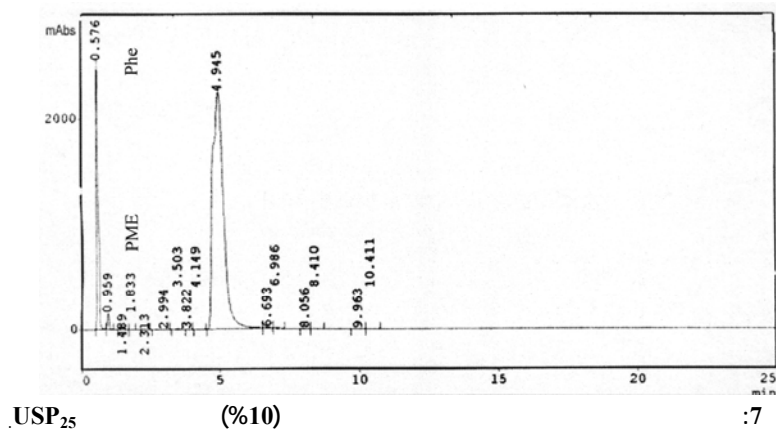
:6

APM

- β

25

205



.[[- 8] [- Specificity

% 102.27 Recovery

Relative Error

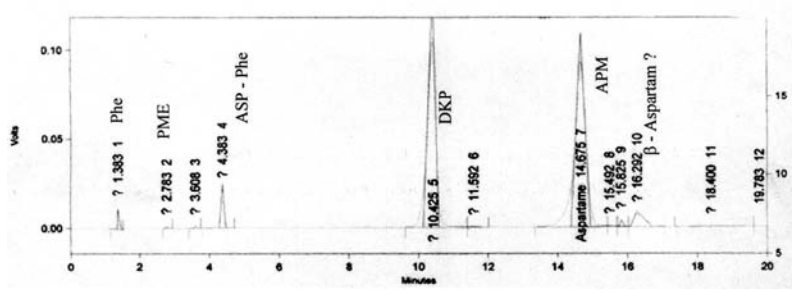
RE = 4.78

% 98.75 Precision Limit of detection

Standard deviation

.SD = 1.784 Retention time

8]) .RT = 14.48



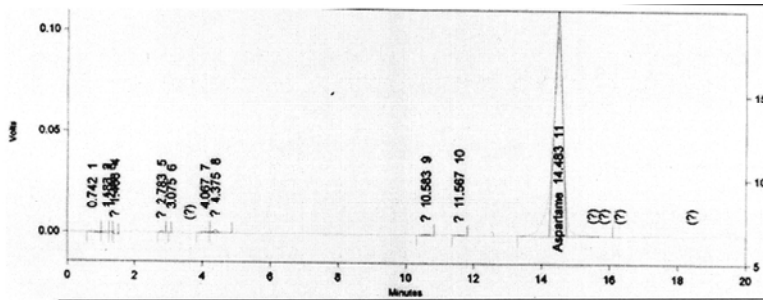
: - 8

% 3 RSD

UV-Vis (210nm) Pk#	Name	Retention Time	Area	Height	Height Percent	Width at 5% height
1	?	1.383	48394	11888	4.87	0.16
2	?	2.783	4784	1001	0.41	0.17
3	?	3.608	6123	1091	0.45	0.21
4	?	4.383	155901	21345	8.75	0.29
5	?	10.425	1866417	104283	42.74	0.64
6	?	11.592	4000	229	0.09	0.47
7	Aspartame	14.675	2159682	92896	38.07	0.94
8	?	15.492	7133	486	0.20	0.00
9	?	15.825	23471	3268	1.34	0.00
10	?	16.292	170583	7132	2.92	0.86
11	?	18.400	21010	342	0.14	0.00

: - 8

% 2 RSD



(100.56
 %) = Intermediate precision
 .SD = 2.15

 %2 t_{exp}
 / 0.1

Combined % 95

 uncertainty t_{exp} = : n - 1
 .% 2.82 2.15 < t = 2.776

 3

Analytical
 .performance characteristics

.()

) Repeatability
 1.76 (Instrumental Precision
 %

) Reproducibility
 % 2.13 (Precision of the Method

:3

				عدد العينات	خصائص الأداء التحليلي
لم يلاحظ أن للسواغات أو منتجات التدرج استجابة عند زمن احتباس العياري الفرق بين مجموعتين أقل من 2 %				7	الانتقائية Selectivity
Slope of Reg. Line		معامل الارتباط (r)		5	Linear and working Range المجال الخطي والعملية للمادة الأولية
2147193		0.9998			
% البقية % Regional	عامل البقية RF Residual Factor	معامل الاستجابة KF Response Factor			
-	18307	4837979			
1.2 - 0.8 مع / مل		مجال خطية الطريقة			
الاسترداد Recovery	الخطأ النسبي Re. Error	وسطي القياسات Means-Av.	القيمة المرجعية References Value	3	المضبوطة Accuracy استرداد من الشوائب أو منتجات التدرج، السواغات
102.27	4.78	98.75	2128337		
حدود الثقة Confidence Limits	الانحراف المعياري النسبي	الانحراف المعياري SD	وسطي القياسات Means-Av.	9	التكرارية Precision - Repeatability
1.72	1.764	1.784	101.09		
2.08	2.13	2.15	100.56	9	قابلية الإعادة (Precision - Reproducibility) intermediate Precision
نتيجة بعد التغير	نتيجة قبل التغير	المتغير Variable: Flow Rate ml / min		1	المتانة Robustness
98.17	99.35	0.9			
100.23	99.35	1.1			
% 2.82					الارتباب الكلي Combined uncertainty

)
(
. %2 / 2 / 0.2

Photodiode array

-β

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