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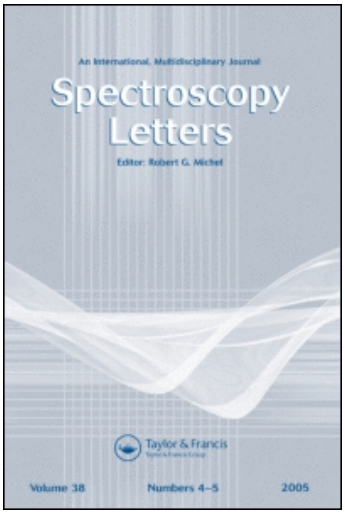

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Derivative Spectroscopy in Conjunction with Thin Layer Chromatography Applied for Determination of Mixtures of Amino Acids in Baby Food.

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Derivative Spectroscopy in Conjunction with Thin Layer Chromatography Applied for Determination of Mixtures of Amino Acids in Baby Food.

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Abstract :

The preparative thin layer chromatography using silica gel and solvent systems n. butanol : acetic acid: water (4 : 1 : 1 V/V) and 96% ethanol : water (70 : 30 v/v) were used to separate the amino acids mixture into groups that can be eluted and one or two of amino acids can be determined by derivative spectroscopy. This was done for determining histidine in presence of cystine, arginine in presence of lysine and ornithine, tryptophan in presence of tyrosine and phenylalanin, and methionine in presence of aspartic acid. The proposed method is applied for determination of these amino acids in baby food, Neslac, Dialac and Nestogen. The statistical analysis of the results were found to be good and in agreement with the ion exchange chromatographic method.

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Introduction :-

Several spectrophotometric methods were used to determine either the individual amino acids or group of the amino acids in mixture however the use of thin layer chromatography in conjunction with the spectrophotometry

facilitate many difficulties that face the determination of the amino acids spectrophotometrically. For thin layer chromatography various reagents has been suggested for revealing the amino acids among these are ninhydrin (1-7). The different colorimetric and spectrophotometric methods that has been revised are colorimetric method through copper complex formation (8), Spectrophotometric method by reaction with 1- fluoro- 2.4-dinitrobenzene (9) , zero-and second order derivative spectroscopy is used after gradient elution from reverse phase short microbore columns (10-11) digestion and colour formation with malachite green, (12) colorimetry condensation with acetylacetone-formaldehyde, (13) spectrophatometry measure ofter reaction with furfuraldehyde (14), spectrophotometry by reaction of amino-acids with p-benzoquinone (15) and flow injection spectrophotometry (16,17). This paper describe the use of The first and the second derivative spectrophotometry for the assay and determination of histidine, arginine, tryptophan and methionine each in presence of other amino acids ($dA/d\lambda$) was also applied for determining tryptophan in presence of tyrosine and phenylalanine applying the zero crossing method of measurement. The preparative thin layer chromatography is used to separate the different amino acids mixtures in groups that is histdine and cystine (group I) arginine, Lysine and ornithine (group II) tryptophan, tyrosine and phenylalanine (group III)and Methionine and aspartic acid (group IV).

Then the both first and second derivative spectroscopy were used to determine one or two of the isolated separated prepared groups. The

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method although take long time for the assay but is found to be in excellent agreement with ion-exchange chromatographic method and can be used in laboratory that dose not have the amino-acid analyzer apparatus also the method shows good results when applied for the acid protein hydrolysate that come from baby food It was found that both the qualitative and quantitative determination or assay of the amino acids hydrolysate shows good results agree with the results of the amino-acid analyzer.

Experimental

Apparatus :-

A schimadzu-260-U.V. Spectrophotometer-Self Recording -double beam-computerized-with 1 cm quartz cuvettes was used. Suitable setting were : scan speed 40 nm^{-1}. chart speed 60 $nm\text{-}min^{-1}$. and slitwidth 2 nm.

- The apparatus can be setted in first and second derivatives mode. Rotatory evaporator-Thin Layer chromatographic plates silica. gel spreaded on aluminum sheet, fluka ready prepared.
- Jars for thin layer chromatography developments.
- Ion exchange chromatography-Amino-acid analyzer.
- Fine calibrated syringe graduated 0.01 ml. of 1.0 ml volume
- **Materials :-**
- Histidine Hydrochloride, Cystine, Arginine, Lysine, Ornithine, Tryptophan, Phenylalanine, Tyrosine, Methionine, Aspartic acid and Hydrochloric acid were supplieded by B.D.H. Analytical Standard grade chemical were used.
- All solvents used were of analytical grade.
- All baby food are collected from marked Neslac (prepared in the Netherlands by Nestle Nederfan V., Amsterdam. Switzerland.) Dialac. (N.V.Nutricia. Zoetermer-Holland.), Nestogen (switzerland).

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Standard Solutions :-

1- Aqueous stock solutions were prepared, containing 12 mg% of Histidine monohydrochloride 24 mg% of cystine, 17 mg % Arginine, 13 mg % of ornithine, 14 mg % of Lysine, 14 mg % of Methionine, 13 mg of Aspartic acid in distilled water, 17 mg % of Tryptophan, 16 mg % of Tyrosine and 18 mg % of phenylalanine in o.1N Hcl.

- Working solutions were prepared by pipetting separately 1.0 -10.0 mL aliquots of histidine, 1.0-8.0 mL aliquots of arginine, 3.0-11.0 mL aliquots of tryptophan and 4.0-19.0 mL aliquots of methionine stock solutions into 50 mL volumetric flasks and completing to volume with distilled water.

2- Laboratory prepared standard mixures.

Mixture (1) :

Suitable aliquots of histidine and cystine stock solutions were piptted into 50 mL Volumetric flasks and completed to Volume with distilled water to give different dilution of 1:1 mixture.

Mixture (2) :

Stock solution were pipetted into 50 mL volumetric flasks and completed to volume with distilled water to give different dilutions of mixture.

Mixture (3):

Suitable aliquots of tryptophan, tyrosine and phenylalanine stock solution were pipetted into 50 mL volumtric flasks and completed to volume with distilled water to give different dilution of (1:1:1) mixture.

Mixture (4):

Suitable aliquots of methionine and aspartic acid stock solutions were pipetted into 50 mL volumetric flasks and completed to volume with distilled water to give different dilutions of (1:1) mixture.

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Baby foods :-

A- preparations of acid protein hydrolysates for chromatography

A weigh of the powder equivalent of about 100 mg proteing is mixed with 12.0 ml of 6 N Hcl in a 50 ml glass ampoule. The ampoule is sealed and then heated at 110 °C in oven for 36 hours, the insoluble black polymer (Humin) is removed by filteration in sintered glass funnel.The solution is then repeatedly evaporated down to dryness under vacum with intermediate addition of water to get rid of excess Hcl. The residue is then dissolvd in about 1.0 mL of water and added to an amberlite 120 H^+ column. the column is then washed with 200 mL water and elution is carried out with 10% HcL. The amino acids solution is collected and repeatedly evaporated to dryness under vaccum with repeated addition of distilled water.

b- preparative thin layer Chromatography the silica gel GF 254 plates dried at 110 °C for 1 hour before use, was used for chramatography. The chromatograms had run in one dimension using solvent systems n-butanol acetic acid: water (4:1:1) V/V for ornithine, arginine and Lysine mixture and 96% ethanol : water (70:30) V/V for tryptophan, phenylalanine, but and tyrosine mixture ( ) methionine, (1.18) aspartic acid mixture, and cystine, histidine mixture.

2- The spot zones were scrapped by a spatule and placed in a conical flask by aid of a small funnel, Hcl solution (10.0ml)were added and left stand in 10 mL volumetric flask for one hour with occassional shaking to completely dissolve the amino acid. Filteration was carried out through an ashless filter paper. Zone free from any spots scrapped and macerated in 10 mL Hcl and used as blank.

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Results and Discussion :-

Since Rf values of the components of the above group are too close to each other in the different solvent system, spectrophotometric measurement are tried to solve this problem.

The U.V. absorption spectra of histidine in presence of cystine (fig.1a), arginine in presence of lysine and ornithine (fig.2a) tryptophan in presence of phenylalanine and tyrosine (fig.3a) and methionine in presence of aspartic acid (fig.4a) are extensively overlapped. Conventional U.V. spectrophotometry can not be used for individual quantitation of the four amino-acids in the mixture form.Histidin,arginine tryptophan and methionine in their mixtures have been suceessfully assayed using first and second derivative spectrophotometry. [Fig. 1.2.3.4(b)]

- The histidine in presence of cystine was detrrmined by second derivative spectroscopy.Arginine in ornithine and lysine was detrrmined by second derivative method,Tryptophan in presence of tyrosine and phenylalanine was determined by first derivative method. [Fig. 1.2.3.4(C)].
- Methionine in presence of aspartic acid was detrrmined by second derivative spectroscopy All the spectroscopic data are Listed in table (1) .
- Applying the thin layer chromatography to the acid protein hydrolysates and subsequent assay of the amino acids histidine arginine and tryptophan in baby food were compared to the results obtaines by amino-acid analyzer. Table (2).
- Table (2) shows the results of six prepared mixtur of amino acid separated by thin Layer chromatography and assayed by derivative spectroscopy.

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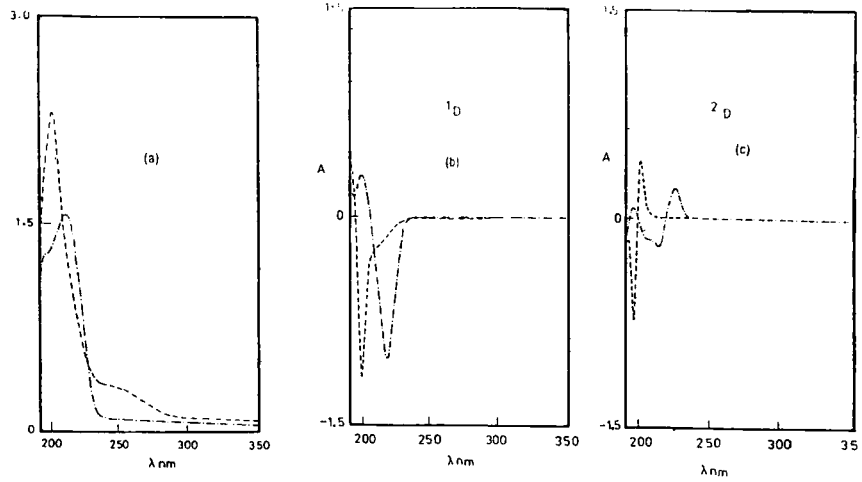

Fig. (1): Zero Order Spectra (a), ^{1}D Spectra (b) and ^{2}D Spectra (c), of 12 mg% Histidine (-·-·) and 24 mg% Cystine (---).

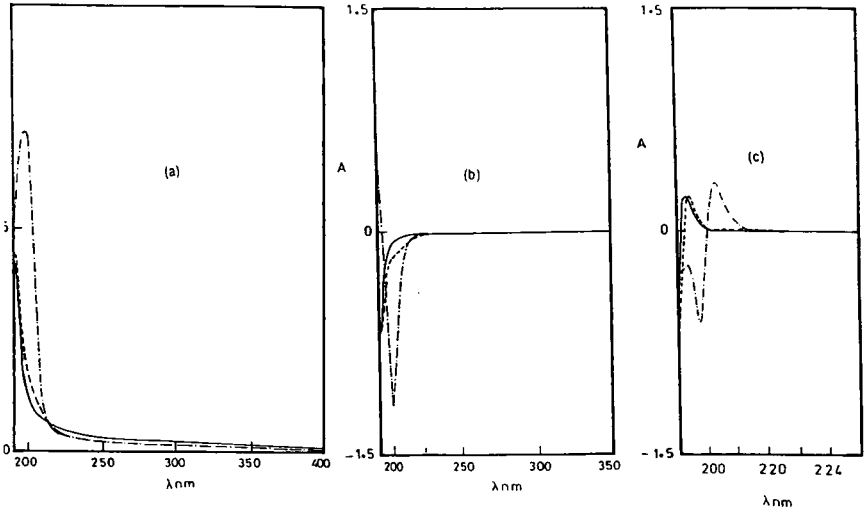

Fig. (2): Zero Order Spectra (a), ^{1}D Spectra (b) and ^{2}D Spectra (c), of 17 mg% Arginine (-·-·) and 14 mg% Lysine (---) and 13 mg % Ornithine (—).

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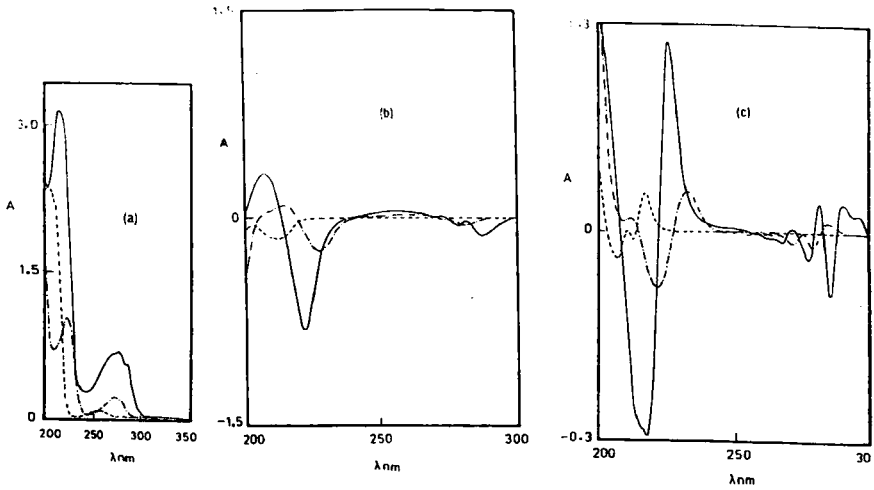

Fig. (3): Zero order spectra (a), ^{1}D Spectra (b) and ^{2}D Spectra (c), of :
(a) 17 mg% Tryptophan (—), 16 mg% Phenylalanine (---) and 18 mg % Tyrosine (-·-·).
(b) 1.02 mg% Tryptophan (—), 1.28 mg% Phenylalanine (---) and 1.26 mg % Tyrosine (-·-·).
(c) 1.02 mg% Tryptophan (—), 1.28 mg% Phenylalanine (---) and 1.26 mg % Tyrosine (-·-·).

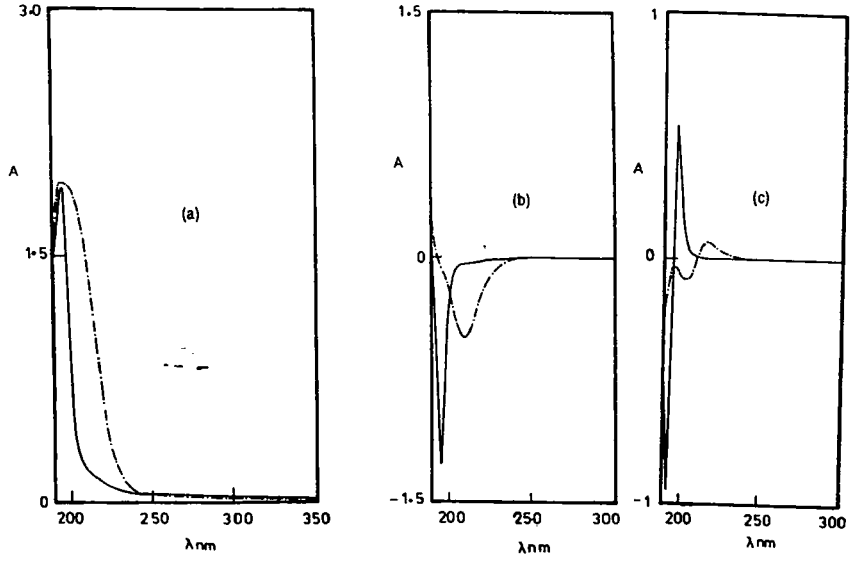

Fig. (4): Zero Order Spectra (a), ^{1}D Spectra (b) and ^{2}D Spectra (c), of 14 mg% Methionine (-·-) and 13 mg% Aspartic Acid (—).

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Table (1) Spectroscopic Data of the amino-acids and its linear Equations of the proposed method utilizing derivative spectroscopy and the results of the authentic samples of amino acids

Components of amino acids & its linear regression equations	Measurements			Concentration range mg %	% recovery	Variance	S.D.	S.E.
	Derivative number	Wavelength	Blank					
Histidine C = 60.164 x A+3.6 x 10^{-3} where R = 0.998	D^2 Second derivative	225 nm	dist. water	0.24 - 2.4	100.1	1.84	1.36	0.453
Arginine C = 24.503 x A - 218 x 10^{-3} where R = 0.998	D^2 Second derivative	203 nm	dist. water	0.34 - 2.72	99.5	1.89	1.70	0.694
Tryptophan C = 7.445 x A+ 0.112 where R = 0.996	D^2 Second derivative	289 nm	0.1 N Hcl	1.02 - 3.74	99.6	0.23	0.48	0.214
C = 24.459 x A - 0.04 where R = 0.999		281 nm	0.1 N Hcl	1.02 - 3.74	99.2	1.34	1.16	0.518
C = 25.308 x A - 0.04 where R = 0.997	D1 First derivative	290 nm	0.1 N Hcl	1.02 - 3.74	99.8	6.1	2.48	1.109
Methionine C = 16.86 A+ 0.476	$-^2$ D Second	215 nm	water	1.02 - 3.74	99.7	3.8	1.95	0.796

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Table (2) Results assay of amino acids in laboratory prepared mixtures and results assay of amino acids in different types of baby foods by derivative spectrophotometric method in conjunction of T.L.C. (A) and Ion-exchange chromatography method (B)

Amino acid	Method	Concentration range mg %	% recovery	Variance	S.D.	S.E.	Baby food name	found in mg	
								A	B
Histidine	^{2} D, λ = 225 nm	0.24 - 2.4	99.48	1.23	1.11	0.45	Neslac Dialac Nestogen	1.131 1.05 1.65	1.35 1.25 1.50
Arginine	^{2} D, λ = 203 nm	0.34-2.72	98.16	1.02	1.01	0.41	Neslac Dialac Nestogen	2.30 2.53 1.65	2.09 2.38 1.50
Tryptophan	1 D, λ = 289 nm ^{2} D, λ = 281 nm ^{2} D, λ = 290 nm	1.02 - 3.74	97.4 98.2 98.4	1.82 0.54 1.02	1.35 0.74 1.01	0.6 0.3 0.4			
Methionine	^{2} D, λ = 215 nm	1.12 - 5.32	97.6	0.193	0.44	0.15			

However the low concentration range of the amino acids mixtures used before chromatography due to the capacity of the layer of adsorbent is low that can carry the amino acids by the solvent developer in comparison to impregenated kieselguhr G layers with 0.1 M of sodium hydrogen phosphate (NaHPO4) bufer (PH=5) that shows capacity of about 25 mg of any individual amino acid (19).

References

1- Stahl, E. " Thin Layer Chromatography " A laboratory Hand book., springer , Berlin, 406, (1965).

2- Adams, H.W. and Stuart, H.G.; Analyst, 76.553, (1951).

3- Kewerau, E. and Wieland, T.; Nature, 168,77, (1951).

4- Jeanes, A.,Wise. C.S. and Dinsler. R.J.; Anal. Chem. 23,415,(1951).

5- Fitzpatrick. W.H., Science, 109,459, (1949).

6- Trall.W. canna., R.R.;J. Biol. Chem,., 200, 803(1953).

7- Lamothe. P.J. and Mccormick. P.G. Anal-Chem.45 (11), 1906(1973).

8- Speis. J,R. j. Biol, Chem,. 195,65,(1952).

9- Comors, R.A. and Wong. M.P.j. Pharm. Sci . 68.11.1470, (1979).

10- Grego. B, Nice.E.C. and simpson. R.J.; J.Chromatog. 352; 356 (1986).

11- Burguera. J.L.; Burguera. M.; dela Guardia M. and salvador.A.; Anal. Chim. Acta. 261,23.(1991).

12 Devani. M.B., shishoo, C.J., shah., S.A soni, K.P. and shah. R.S.j. Chromatog. 537,494 (1991) .

13- Idem Indian. J. Pharm. Sci., 54 (5),201 (1992).

14- Ibolya M.P. and Margit. P.S.; Anal. chim. Acta., 257 (2), 209, (1992).

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15- Zoia. D.A.M., Barreto. W.J., santas, N.J. and Enda, A.S.;Anal. Chim, Acta, 277 89 , (1993).

16- Laverta, Z.L . and Martinez.c. ; J. Anal., Chim. Acta. 281, 601,(1993).

17- Sauria. J. and Hernanday-Cassou. S. Anal. Chim. Acta., 281,593, (1993).

18- A.A. El-Kheir, M.M.F. Metwally and M.M.Ayad Egypt-J. food. Sci., 19, (1-2),PP 41-51,(1991).

19- K.Kvingested. Acta. Chem ,. Scand. 18,2399 (1964).

20- joint FAO-WAO, Expert Consultation protein Quality evaluation, food and Nutrition paper, 151, food agriculture organization of the united Nations Rome (1991).

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