# Analysis of organic compound by Paper Chromatography: Review

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**Abstract:**

Paper chromatography technique has been extensively used by various workers in the past for the analysis of free amino acids in the body parts or entire body of various organisms, to observe their variation among the various developmental stages or in various sexes or among the various species and genera at the biochemical level.

**Key Words: Chromatography, Biochemical, Amino acid, Grasshopper**

**Review :**

To the best of the authors knowledge very little work of this type has been done earlier on the orthopterans. Pant and Aggarwal (1963b) studied the FAA compostion of the haemolymph of *Acrida exaltata* (Orthoptera), *Poecilocerus pictus* (Orthoptera) and *Bellastoma* (Hemiptera). 17 amino acids namely alpha-alanine, Beta-alanine, aspartic acid, cysteic acid, glutamic acid, glutamine, histidine, lysine, ornithine, tyrosine, valine, phenylalanine, proline, ser-ine, glycine, isoleucine, and tryptophan were shown to be present in the adults of *A.exaltata*. They detected 17 FAA in the nymph and 19 FAA in the haemolymph of *Poecilocerus pictus.* They discussed the presence of these free amino acids in relation to the role played by them in various metabolic functions. However, Bellamy (1958) in *Schistocerca greqaria* noted that glutamic acid is more concentrated in the head *than* in any other tissue. Benassi and Columbo (1961) had shown that the total concentration of amino acids remain constant during the larval period in *S. gregaria.*

Lot of work is available in this field but on other representatives of insects than the order Orthoptera. In the flies and mosquitoes, various workers have analysed their various developmental stages for FAA in them (Agrell, 1949; Hicks and Ellis, 1951; Hodorn and Mitchell, 1951; Chen, 1960; Dang and Pant, 1964; Chaput and Lilies, 1969; Singh et *al.* 1971, 1973; Cavalloro and Phillippe, 1974; Sarin, 1975; Sidhu a-nd Kang, 1979 and Gakhar and Nagpal, 1989).

Earlier workers have analysed the FAA in the whole body of the males and females of various insect species to see the differences in them *(Auclair* and Dubreuil, 1952; Fox, 1954; Kaplan and Others, 1957; Chen, 1958; Chen and Diex, 1961; Duffy, 1964 and Thakare et *al.*, 1976).

Chromatography is a technique which helps in the separation of the chemical ingredients of a biological sample. The importance lies primarily in its use as an analytical tool. Paper chromatography did not become much popular until the work of Consden *et al.* (1944) who not only introduced, but also demonstrated its applications.

Bacteriologists, geneticists, botanists, zoologists and a variety of other workers have employed paper chromatography for the study of proteins, amino acids, carbohydrates, steroids, antibiotics, vitamins and many other substances in the biological systems. Entomologists and many others interested in the biochemistry and nutrition of the organisms have employed this method for the purpose of studying the

amino acids of the insects because of its simplicity, versatality, reproductibility and the possibility of identifying impure substances,

Adriano *et al.* (1953), Kirk et *al*. (1954) & Micks (1956) have applied paper chromatography as a tool in taxonomy and population genetic studies. The various chromatographic techniques have also provided their usefulness in the determination of various organic substances in the biological systems to a standard accuracy by which differences or similarities in the free amino acids can be observed at intraspecific as well as interspecific levels. Buzzarti (1953), Fox (1954, 56) and Kaplan *et al.* (1957) are the few taxonomists who made the use of this technique. Moreover, from the chromatography of the developmental stages, the interrelationships of the species can be well established.

Dent (1947) was the first geneticist who introduced the paper chromatography in genetic analysis. Kodani (1948), Blumel and Kirby (1948) Cark and Ball (1951), Scossiroli and Rasmussen (1954), Mukerjee and Strohmann (1962) and Bawa *et al*. (1974) are the other geneticists who employed this technique in their research work.

The major ontogenic events such as growth, moulting, population etc. are basically the result of the process of cell differentiation which in turn involves the synthesis of proteins. It is in this respect that the importance of free amino acids becomes evident as they are the building blocks of the proteins and are used in their synthesis as coded by the genetic constitution of an organism.

The concentration of the free amino acids in the insect body is higher as compared to the other animals. The higher concentration of the free amino acids is believed to play an important role in osmoregulation as suggested by Bishop *et. al*. (1926) and Beadle & Shaw (1950). Besides, buffering of the blood, the main function of the free amino acids is in the protein synthesis or in metabolism. Quite a number of amino acids are known which play a role in the synthesis of cuticle i.e. cuticular proteins, chitins, polyphenols etc. as reported by Pant and Aggarwal (1963^6) According to Pant and Varma (1974), the free amino acids play an important role in metamorphosis and detoxification mechanism in the insects similar to the higher animals. Similar reports have been also given by Shyamala (1964).

Chen (1958), Kaplan et al. (1958), Fox (1956), Duffy (1964), Thayer and Terzian (1970) have reported that there exists a difference between the amount and kind of ninhydrin positive substances in the two sexes of the various insects. Pratt (1950), Micks and Ellis (1951) have also attempted to analyse the free amino acids in the adult insects in order to differentiate the sexes on the basis of chromatographic analysis.

The literature thus reveals that chromatographic analysis of free amino acids in the bodies of various insects constitute a promising field of research. It is believed that the present work which includes the paper chromatographic analysis of the free amino acids in the whole bodies of the nymphs and the adults and in the different body parts of the three adult orthopteran species viz. *Acrida luqubris. A. giqantia and Chrotogonus trachypterus* would add to our existing scientific information in this area of research. It is noteworthy to mention that earlier, no work has been done on this aspect in these three species.

Consden *et al*. (1944) were the pioneer workers in the modern paper chromatographic technique. They started this technique in Britain. The underlying principle is that the substances to be analysed distribute themselves between stationary and mobile phases in proportion which vary from substance to substance. The use of one stationary phase and one mobile phase is the common feature of all the chromatographic techniques. Because of its simplicity, versatality and reproductibility the .technique of paper chromatography has been employed by many zoologists, geneticists, botanists, chemists and

pharmacologists.

Dent (1947) performed the experiments of paper partition chromatography in the genetic investigations, and is considered to be the .first geneticist who introduced chromatography in genetic studies.

Agrell (1949) studied the occurrence and metabolism of amino acids during the insect metamorphosis. He found that there is a slight reduction in the total concentration of amino acids at the initiation of pupal stages. The concentration rises in the early mid pupal stage and again during the later half of the pupal period of Calliphora.

Finlayson and Hamer (1949) reported the presence of alanine, aspartic acid, glycine, histidine, leucine, lysine, phenylalanine, proline, serine, tyrosine and valine in the haemolymph of Calliphora larva,

Pratt (1950) analysed ten amino acids in the blood of the seven species of the insects by paper partition chromatography.

Clark and Ball (1951) analysed the free amino acids present in the whole body of *Culex tarsalis*. *C. stigmatosoma*, *Aedes varipalpus* and *Culiseta incidens* by 2-diaensional paper chromatography. The amino acids alanine, arginine, glycine, glutamic ac^-d/ phenylalanine, proline, serine, methionine, threonine, valine and glutamine were found in all the cases. Cystine, histidine aspartic acid, lysine, tryptophan and gamma-amino-n-butyric acid were found in many but not in all the cases. Tyrosine which is considered the building block in the tanning of the insect article was found only in the *Aedes varipalpus*.

Hodorn and Mitchell (1951) studied the various developmental stages, tike different body parts and the organs of *Drosophila melanogaster* by employing the paper chromatographic technique. They observed various significant differences in size and intensity of the colour of the spots in different tissues and genotypes thus showing a new field in the study of biochemical genetics. The maximum concentration of amino acids was attained at the third instar larval stage. Moreover, they found the maximum concentration of amino acids in the thorax and the minimum concentration i.e. only traces in the malpighian tubules.

Micks and Ellis (1951) qualitatively analysed the free amino acids in the mosquitoes. They found that three genera of mosquitoes. (Culex. Aedes and Anopheles) could be readily distinguished by marked quantitative differences in the amino acid levels. Chromatograms of the larvae and adults of *Culex molestus*, *C.fatigans* and *Aedes quadrimaculatus*, although similar, exhibited quantitative differences in their ninyhydrin positive materials. Both the sexes presented the same chromatographic patterns.

Ball (1952) analysed the free amino acids in whole bodies of culicid mosquitoes and found that the three species of *Culex* mosquitoes (*fatigans*, *pipiens* and *molestus*) presented quantitatively different chromatograms of the flourescent substances.

Micks and Ellis (1952) analysed the free amino acids in the eggs, larvae, pupae and adult mosquitoes. The highest concentration of amino acids was reported in the larvae followed closely by the adult individuals. The larval period is thus considered the period of maximum growth.

Auclair and Dubreuil (1952) were able to quantitatively detect the differences of the free amino acids and the peptides in the two sexes of D. melanocgaster, but they were unable to identify the additional spot which was present only in the males.

Buzzarti-Traverso (1953) reported the biochemical constitution of an organism or its parts as revealed by paper-chromatography technique. It was exceedingly constant and largely independent of the dietary and environmental conditions. They also employed the paper partition chromatography in the taxonomic studies.

Hackmann (1953) reported the presence of tyrosine in the proteins of insect cuticle. Tyrosine serves as a substrate for the tanning reaction of the larval cuticle at the time of puparium formation.

Giri (1953) applied the circular chromatographic technique for the separation, identification and qualitative estimation of most of the amino acids present in the protein hydrolysates.

Scossiroli and Rasmussen (1954) employed the 2-dimensional paper chromatography to determine the flourescent compounds in the hybrids and the parental species of *Drosophila*. They observed that the hybrids show a peculiar flourescent pattern characterized by the traits of both the parental species. Some of the spots were found to have increased in size and intensity of the fluorescence over those obtained from the parents.

Rasussen (1954) in fact studied twenty different species of the families Drosophilidae and phoridae in order to compare the biochemical similarity with the taxonomic relationship. He observed that there is no difference of ninhydrin positive pattern among the various species of the drosophilidae but differences have been found between the members of family phoridae on one hand an Drosophilidae on the other. Thus the grouping of the species according to the biochemical similarities correspond to the taxonomic grouping on the basis of morphological characters. Some spots so revealed by the paper chromatography are suitable for distinguishing the established taxonomic levels (families, genera, subgenera, groups and subgroups).

Kirk et al. (1954) chromatographed fresh tissue extracts of the foot muscle of seven species of land snails for their taxonomic study. They observed that each species given a pattern which can be distinguished clearly from that of the others. Variations in the age, geographical locality and three different diets under different laboratory conditions caused no significant differences in the characteristics pattern observed by them.

Fox (1954) the first who identified twenty two ninhydrin positive substances in both the sexes of *D.melanogaster*. Out of these eighteen were found to be free amino acids eg. Aspartic acid, glutamic acid sarine, cystine, ornithine, glycine, lysine, threonine glutamine, alanine, arginine, tyrosine, histidine or citrulline, valline, nor-valine, methionine, tryptothan and leucine. He also reported the presence of a sex peptide which was present only in the males

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Fox (1956) investigated the entire male and female individuals of Drosophila with the view to study chromatographic differences and the role of X and Y chromosomes. According to his findings, the chromatographic differences between the two sexes are attributed to the number of X-chromosomes and the differences in the balance between the X-chromosomes and the autosomes, which are similar to those involved in the sex-determining mechanism. Therefore, the sex-determining mechanism may by itself be responsible for the chromatographic differences.

Hackmann, (1956) studied the chromatographic differences in the free amino acid content of the blood of blowfly *Calliphora angler*. According to him the amino acids present in the blood of insects play an important role in the metamorphosis.

Micks (1956) applied, paper partition chromatographic techniques in insect taxonomy. These methods were applied to the whole specimen as well as extracts of Hemiptera, Orthoptera and Diptera. One dimensional chromatograms revealed qualitative differences between these three orders in their respective patterns of ninhydrin-positive substances. Three genera of mosquitoes *Culex. Anopheles* and *Aedes*) could be readily distinguished by marked quantitative differences in their amino acids levels. Chromatograms of larvae and adults of *Culex molestus. C.fatigans and Aedes quadrimaculatus* although similar, exhibited quantitative differences in their ninyhdrin-positive materials. Both the sexes presented essentially the same chromatographic patterns. The three species of Culex as well as Aedes presented quantitatively different chromatograms of flourescent substances.

Kaplan and others (1957) studied the free amino acids pattern of different life stages of the wild type and several mutant strains of *D.melanogaster* by 2-dimensional paper chroaiatography. They observed that methionine concentration is higher in the females than in the males. They further reported that glutamic acid, taurine, alanine, glutamine and proline are present in higher concentration whereas leucine, histidine, aspartic acid serine, glycine and valine are found in low concentration,

Bellamy (1958) noted that in Schistocerca gregaria glutamic acid is more concentrated in the head than in any other tissue.

Hoenigsberg and Castiglioni (1958) studied the biochemical differences between inbred and outbred lines of *D. melanogaster* by paper partition chromatography. They found a constant and a typical pattern of nine flourescent compounds found in the Drosophila ageing stocks and its absence in the inbred lines.

Chatterji and Sarup (1960) in *Rhizopertha dominica Calandra oryzae* and *Anthrenus vorax* have shown the absence of phenylalanine which is one of the essential amino acids in the diet of larval stages of insects.

Chen (1960) demonstrated that a number of free amino acids such as arginine, cystine, glycine, proline, tryptophan, tyrosine and phenylalanine are indispensable for moulting, differentiation, pupation and adult emergence.

Florkin (1960) reported that the insects are characteristic in having very high concentration of free amino acids in comparison to the other animals.

Benassi and Columbo (1961) reported that the total concentration of amino acids remain constant during the larval period in Schistocerca.

Chatterji *et al*. (1961) showed that the different stages of Trogoderma granarium bear fifteen types of amino acids by unidimensional chromatographic technique.

Chen and Diem (1961) following Fox and others (1959) reported a free ninhydrin reacting component in the paragonia of the adult males in *D.*melanodaster by employing two-dimensional chromatographic technique. Obviously this ninhydrin-positive substance corresponds to the sex-peptide reported by Fox (1956). Aspartic acid, glutamic acid, glycine, alpha-alanine, valine and leucine are located by hydrolysis of the sex-peptide. This sex-peptide was found to be absent or in very small quantity when

present in *Culex pipiens and Culex fatigans.*

Mukherjee and Strohmann (1962) made an attempt to make a comparative study of the chromatographic patterns of the heterozygous and homozygous condition of the mutant and that of the wild type. They concluded that the homozygous vestigial males and the females differ from the heterozygous and normal flies. The difference is attributed to the number and the colour of the spots and their Rf values. Both the flourescent and the U.V. spot absorption patterns were obtained in all the cases. They further reported that the $R_f$ values of the flourescent spots of a given genotype vary considerably in both the sexes.

Pant and Aggarwal (1963b) studied the free amtno acids composition of some hemi and holometabolous insect haemolymph quantitatively by paper partition chromatography. Although each sample of haemolymph has its own pattern the presence of alpha-aminoisobutyric acid, homoarginine and hydroxyproline seem to be characteristic of the haemolymph of *Attacus ricini.* The presence of more than one guanine derivative besides arginine has been noted in the haemolymph.

Pant and Kapoor (1963b) made the qualitative analysis in the developmental stages of normal and aposymbiotic *Iasioderma serricorne*. They found that the tissue extracts of normal larvae contain 19 amino acids.

Duffy (1964) determined the sex differences in three colonized mosquito species. The most striking sex difference observed by him was the presence of larger amount of Beta-alanine in the males than in the females. Males of both *C. pipiens* and *C. molestus* have more lysine plus histidine than the females and the males of *A. aegypti* have more proline and serine than females.

Chen (1962) used the automatic amino acid analyser for the study of the changes in the amino acids and the peptides and the related compound during the post-embryonic development in insects.

Chaput and Lilies (1969) found that in Aedes the total concentration of amino acids is maintained at a constant level during the larval development. They separated 36-ninhydrin positive compounds on the chromatograns of deproteinised extracts of insects. Glutamic acid, alanine, tyrosine and arginine consistently occurred in higher concentration among the free amino acids whereas among the peptide bound amino acids, glutamic and aspartic acid were principle. Inspite of the various fluctuations no definite pattern emerged from the study. The occurrence of citrulline and urea during larval stages suggests that urea may be the important excretory product during this stage.

Singh *et al*. (1973) have recorded the occurrence of aspartic acid, glutamic acid, tryptophan / methonine, valine, leucine/isoleucine, threonine, arginine, alanine, glycine, serine, proline, cysteine, histidine, lysine and citrulline in 'the developmental stages of *Leucinodes orbanalis.*

Rakshpal and Singh (1973) reported the presence of alanine, glutamic acid, glycine, proline and serine in all the tissues of *Periplaneta americana.*

According to Bawa *et al*. (1974), paper chromatographic studies of the free amino acids in the normal and sterile strains of *Callasobruchus maculatus* have not revealed any differences whatsoever. Both the strains have histidine arginine, aspartic acid, serine, glycine, threonine, alanine, leucine and phenylalanine.

Cavalloro and Philippe (1974) carried out the studies using thin layer 2-dimensional chromatography system to determine free amino acids in the larval and adults of *Dacusolea* collected in natural environment. Results were discussed in relation to the well known free amino acids of olives and the

total amino acids of third instar larvae grown on an artificial medium. Of the 19 amino acids found, 15 proteinic ones were only identified.

Sarin (1975) made the qualitative ananlysis of the free amino acids in the developmental stages of *Albetobius diaperines* (Panzer) and stated that almost all the amino acids present in the adult also occur in the egg.

McGregor and Ionghton (1976) have shown that the changes in the amino acid pool and in the individual amino acids are related to the morphological stages of the embryo.

Thakare et al ( 1976) have identified sixteen amino acids in the haemolymph of dragon fly *Pentala flavescens*. Beta-alanine was higher in the males than in the females. Arginine, leucine, isoleucine, glutamic acid was more in females. The concentration of methionine was lower than the other amino acids in the males but was comparativelt higher in the females. Cystine and Cysteine were not identified.

Dhillon and sidhu (1977) studied the distribution of free amino acids in the various tissues ( haemolymph, ovaries, testis, thoracic muscles and brain) of *Mylabrus pustulata* by Thin Layer Chromatography. The haemolymph contains 16, ovaries 12 while thoracic muscles and brain contain only 4 amino acids.

Sidhu and Kang (1979) analysed the metabolic reserves and pool size of the free amino acids during metamorphosis of *Callasobruchus maculatus* (F.) and also worked on the presence of free amino acids in normal and starved females of this species.

Gakhar and Nagpal (1989) the qualitative and quantitative changes in the free amino acid content in the excreta of different larval instars of Diacirisa *obliquia* a by 2-dimensional paper chromatography. In all 18 free amino acids, have been observed in the instars I and VI. The amino acids aspartic acid/ GABA, leucine/isoleucine, serine and glycine were common in the excreta of all the larval instars. Ten free amino acids aspargine, glutamine, lysine, ornithine, phenylalanine, pro line, tryptophan, tyrosine, threonine and valine disappeared in the instar II and III. GABA and serine were predominant in the excreta of all the larval instars. The change in these have been related to the various physiologcal events during larval growth.

Mathsushima *et al.* (1989) reported the changes in the free amino acids concentration in the tissues of the fresh water pulmonate, *Helisoma duryi* during hypertonic stress. They found that the amino acids alanine, glutamate, aspartate, glutamine, glycine and serine were the major components of the free amino acid pool which increased in response to hypertonic stress.

Virginia Melo-Ruiz *et al.* 2015 studied Chemical composition and amino acids content of five species of edible Grasshoppers from Mexico and found that a high protein content, ranging from 70% to 75%; lipids 4% to 6%; minerals 3% to 5%; fiber 6% to 8% and soluble carbohydrates 6% to 18%, including essential amino acids that play an important role in human metabolism, with tryptophan as limiting factor. The different species of the studied grasshoppers are high in proteins, essential macromolecules for human life that decrease protein-energy malnutrition; the excess of amino acids after releasing the amino group will provide energy, as well as soluble carbohydrates. They also have minerals that are needed to prevent anemia, and fiber, non-digestible carbohydrates essential in a healthy diet. In conclusion, the daily consumption of 5 or 6 grasshoppers is a good source of nutrients that will provide optimal health to population.

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