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Formazan: A New Powerful Route For Thin Layer Chromatographic Analysis of A-Cyano-Ester Pyrethroids

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Abstract

A new thin layer chromatographic method for the detection of aldehydes is reported with special emphasis on analysis of three different commercial formulations of toxic pyrethroid#1 #2 [1,2] pesticides as real samples. Pyrethroids having alpha-cyano ester group undergo alkaline hydrolysis to yield aromatic aldehydes which were converted to hydrazones by reacting them with simple arylhydrazine reagent like phenylhydrazine and the hydrazones thus formed were subsequently converted to brightly coloured and red shifted formazan dyes. This method not only detects aldehydes so formed, but also gives a confirmatory test for their presence and offers much better visual results than what hydrazones can offer.

Keywords: Formazan Dyes, Cypermethrin, Deltamethrin, λ-Cyhalothrin , Chromogenic spray reagent, Detection of Aldehydes and ketones

1. Introduction

Pyrethroid insecticide are those whose structure resembles different types of compound found in some flowers of pyrethrin genus (*Chrysanthemum family*). Pyrethroids shows more insecticidal but comparatively less mammalian toxicity. Due to ban on chlorinated pesticides and due to extreme toxicity of organophosphate pesticides, pyrethroids are now preferred over these two types of pesticides. Some commercial formulations of pesticides also contain a small percentage of pyrethroid with organophosphorus insecticide. For example Chlorpyrifos or Profenofos is mixed with cypermethrinin in many formulations. Due to wide and easy availability of these pesticides, they are often found to be involved in suicidal and accidental poisoning cases. Hence specific and sensitive methods are required for their detection. There are mainly two different approaches for detection of pyrethroids. One approach consists of detection of cyanide (nitrile) which is formed after alkaline hydrolysis of these pesticides#3. The second approach consists of detection of aldehydes which is formed in same course#4. Both the approaches have their strengths and limitations. Aldehyde is an important and versatile functional group in organic chemistry and many compounds belonging to this class are part of our life. cinnamaldehyde, cilantro, and vanillin provides flavours to essential oils. Acetaldehyde is the main metabolite of Ethanol. Retinal is one of the many forms of vitamin A. Due to their important role in industry, their detection and identification is very essential. There are several methods available for detection of these compounds.

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Colorimetric reagents currently available for detection of aldehydes include MBTH reagent which involve formation of hydrazone first and then formazan dye but require presence of an oxidant. Purpald® can detect only aldehydes but not ketones because ketones doesn't gives colored triazine upon air oxidation. Resorcinol is useful giving colour change by condensation reaction, but being Phenolic, needs careful handling. One more widely used technique to detect presence of aldehydes and ketones is to convert them to their hydrazone derivatives by using different arylhydrazines like phenylhydrazine, 2',4' dinitrophenylhydrazine . Generally hydrazones are solid compounds and have different red shifted colour (high λ_{max}) than parent carbonyl compounds. Hence this method is quite useful in analysis of such compounds. Many spot test, visible spectrophotometric and thin-layer chromatographic visualization reagents are based on this technique. However this method has some inherent limitations. The colours of hydrazones formed are mostly yellow to orange or at the most red. The previous works which reports formation of different coloured hydrazones or schiff's bases are based on use of technical standard of analyte. We have observed that some of these reagent do not produce identifiable colour (or colour formation is very dull) with dilute commercial formulation. Such reagents are of little forensic value, as forensic analysis generally involves detection of these available dilute formulations as evidences collected from crime scene or even more dilute samples to be extracted from viscera and body fluids. A reagent which detects technical standard but not dilute commercial formulation may thus prove less effective and dependence on such reagents may give false-negative results. Most of these reagents produce yellow colour with non-technical samples but a red shifted colour with technical standard of analyte. As, many organic compounds of interest may already have faint or bright yellow colour as the case with many pesticides, the formation of similarly coloured yellow hydrazone is generally less desirable. A reagent which converts yellow analyte to a different shade of yellow product will be thus less desirable. In contrast, if given reagent converts colourless or faint yellow analyte to orange, red, purple or blue product (Figure-1), it will be definitely preferred because final product will have completely different colour than analyte. Many analytical chemists emphasize on high λ_{max} of the product formed along with high extinction coefficient, as at high λ_{max} there are less interferences at higher wavelength. High extinction coefficient provides bright products and consequently more sensitivity.

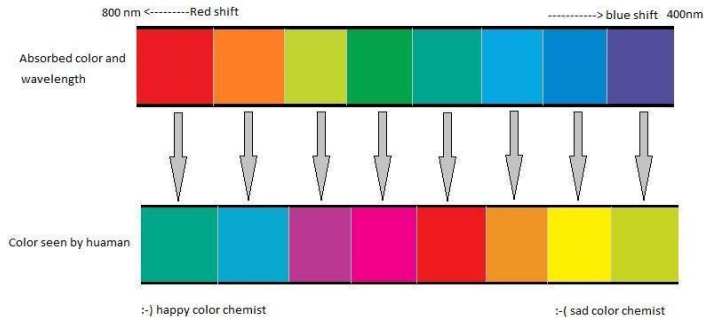

Figure-1: Relation between λ_{max} and observed colour

There are two ways to get this red shifted product. First one is to use reagent with extra conjugation and second one is to use reagent with extra electron donating and accepting group placed properly. The placement of electron releasing or accepting or both types of groups in a reagent as in the case of 2',4' DNP may help sometime to shift the product absorption to red region, but such compound may be itself coloured which is exactly the

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case with 2',4' DNP which is a bright orange solid. When using such coloured reagent, if products do not have much different colour than analytes, there may remain some doubts in testing, especially during spot and wet test. Though solid hydrazones are formed, the sample which already has some turbidity would be little difficult to test. The conversion of colourless analyte and reagent to coloured product with high λ_{max} is something that is desirable during development of an analytical reagent. The problem with coloured reagent thus leaves behind only one way to get more red shifted product and that is to use more conjugated arylhydrazine which itself is not coloured and also do not change its colour in media it is supposed to be used like organic, aqueous, acidic or basic media. Designing such bulky reagent may sometime be difficult for a synthetic chemist, which again adds to the cost of the reagent. Due to all such difficulties it was necessary to find a method which is sensitive but easy to perform during analysis of these carbonyl compounds. We here report a very simple method using easily available analytical reagents to avoid all these difficulties and that is to use two step reactions. It is possible to convert product of reactionsn of aldehyde and aryl hydrazine i.e. the hydrazones, to Formazan dye by just adding or spraying an additional reagent to convert them to highly intense, mostly insoluble dyes called formazan. A brief account of formazan dyes can be found in this paper. While there are many methods to prepare formazan dyes, one popular method involves reaction of aldehydes with aryl hydrazine to give hydrazone and then reacting this hydrazone with an aryl diazonium compound at low temperature and at alkaline pH, giving formazan dye. Formazan dyes have general structure as R-N=N-C=N-NH-R, where R is an aliphatic or aromatic group. Formazan dyes can also be regarded as a special case of azo dyes. These dyes are generally very intense and insoluble and have colours ranging from cherry red to blue. A less intense hydrazones can be converted to more intense formazan dye due to introduction of additional conjugation and extra chromophores. This simultaneously gives more red shifted product and consequently considerable colour difference from original aldehyde as well as hydrazone.

2 Materials and methods

2.1 Chemicals and Reagents

1% Phenylhydrazine hydrochloride in 2N Sodium Hydroxide, 1% Fast Blue salt B in 2N ice cold Sodium Hydroxide, Hexane and chloroform were of solvent grade.

Three commercial liquid formulations of pesticides were purchased from local agriculture service centre with following specifications.

Cypermethrin : 25% , brand name Superkiller-25, Mfg. by Dhanuka Agritech

Deltamethrin : 11% , brand name Decis, Mfg. by Bayer

λ-Cyhalothrin : 2.5% , brand name Lakshya Mfg. by PhytoChem

2.2 Thin-Layer Chromatography

Silica plate was prepared by dissolving Silica Gel-G (Mfg. By Molychem, India Containing CaSO4: 13% and gypsum as binder) in distilled water and uniformly spreading it on a glass plate. The plate was dried in air for 20 minutes or until completely dry and then heated in a hot-air oven to 100°C for another 20 minutes. The plate was then removed and cooled to room temperature and used for spotting with the help of fine capillaries. Ready-made HPTLC silica plates can also be used. Each spot of commercial liquid formulations of Cypermethrin, Deltamethrin and λ-Cyhalothrin were spotted directly (from left to right) on TLC plate without any dilution. A mixture of hexane and chloroform (1:1, by volume) was used as the solvent system which was prepared and kept in a glass chamber for 5 min, for saturation under covered condition. The ascending one-dimensional chromatogram was run to the required height and the distance travelled by the solvent from the base line was noted. The plate was removed and kept in air for 10 min so as it was completely free from mobile phases. The dried plate was then sprayed with freshly prepared alkaline phenylhydrazine solution. The yellowish spots of hydrazones appeared immediately. After 3-5 minutes, which was sufficient time for nearly complete formation of hydrazone, The plate was again sprayed with ice cold alkaline fast blue. The R_f values were calculated for visible spots. R_f values for three spots (considering top front,

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because spots are broad oval rather than circular) were for cypermethrin 6.2 cm/9.5 cm =0.65, for deltamethrin 4.4 cm/9.5 cm = 0.46 and for lambda cyhalothrin 4.6 cm/ 9.5 cm = 0.48

3. Results and Discussion

All three synthetic pyrethroids bearing alpha-cyano ester group under analysis undergo alkaline hydrolysis to give 3-phenoxybenzaldehyde. These aldehydes further condense with hydrazine group of phenylhydrazine giving yellow coloured hydrazones in alkaline condition **(Figure-2)**.

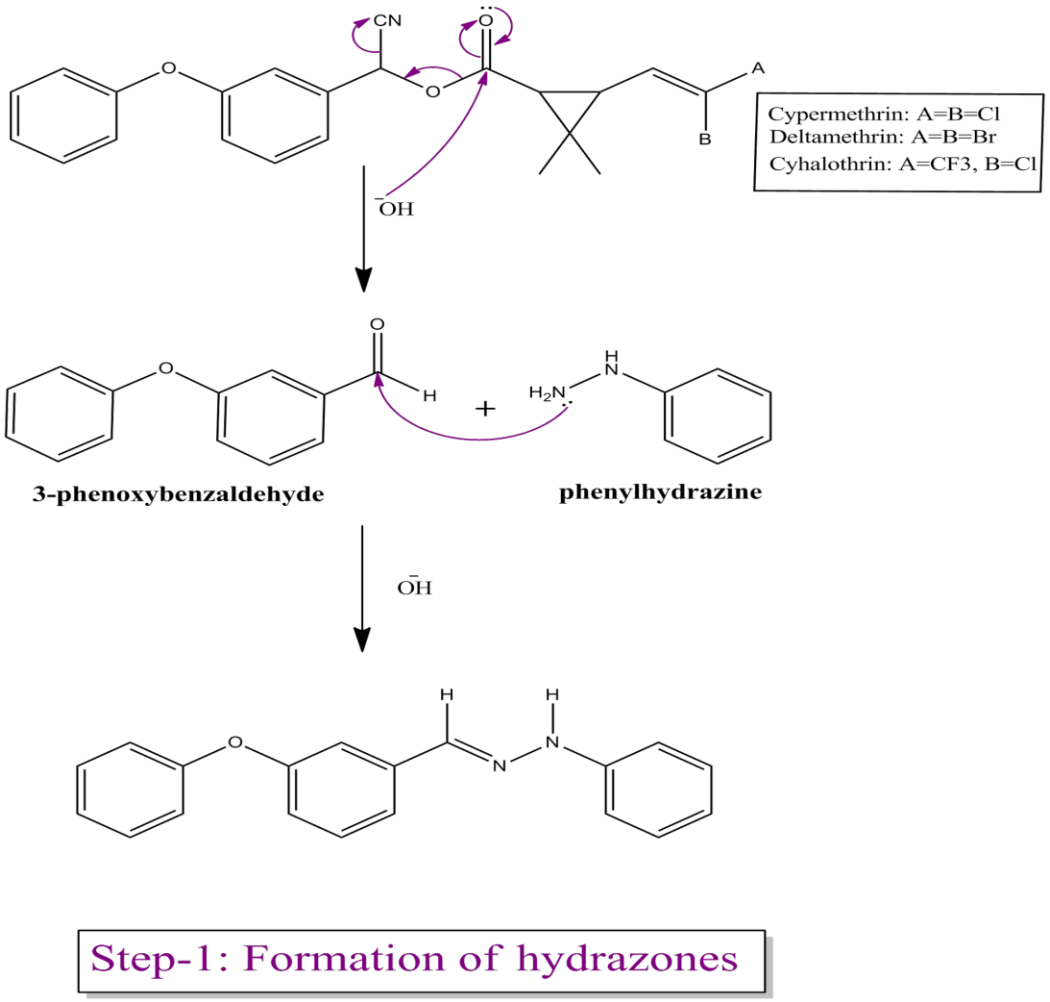

Figure-2: Formation of Hydrazones of 3-phenoxybenzaldehyde and phenylhydrazine

One can easily observe that these hydrazones were not much intense **(Figure-3)** .

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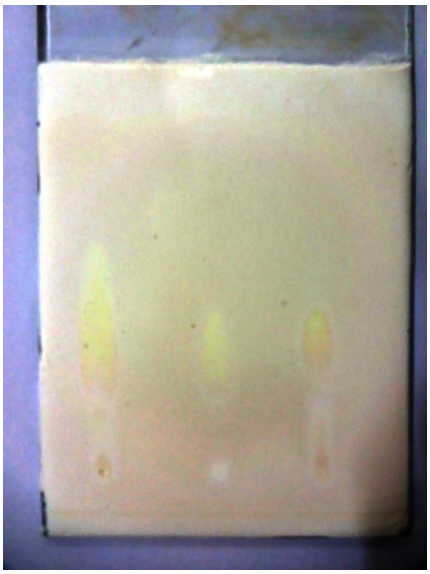

Figure-3: TLC plate showing chromatogram of Hydrazones formed

Their formation is rapid, clear cut, but spots are somewhat dull. It is important to note here that the background i.e. the non-chromatographic zone was also faint yellow in colour and due to loss of contrast, visual effect produce by hydrazones in chromatographic zone is not very impressive. When the plate was sprayed with ice cold alkaline fast blue, the hydrazones attacked the diazo group of fast blue from both the sides giving bis-formazan **(Figure-4)**.

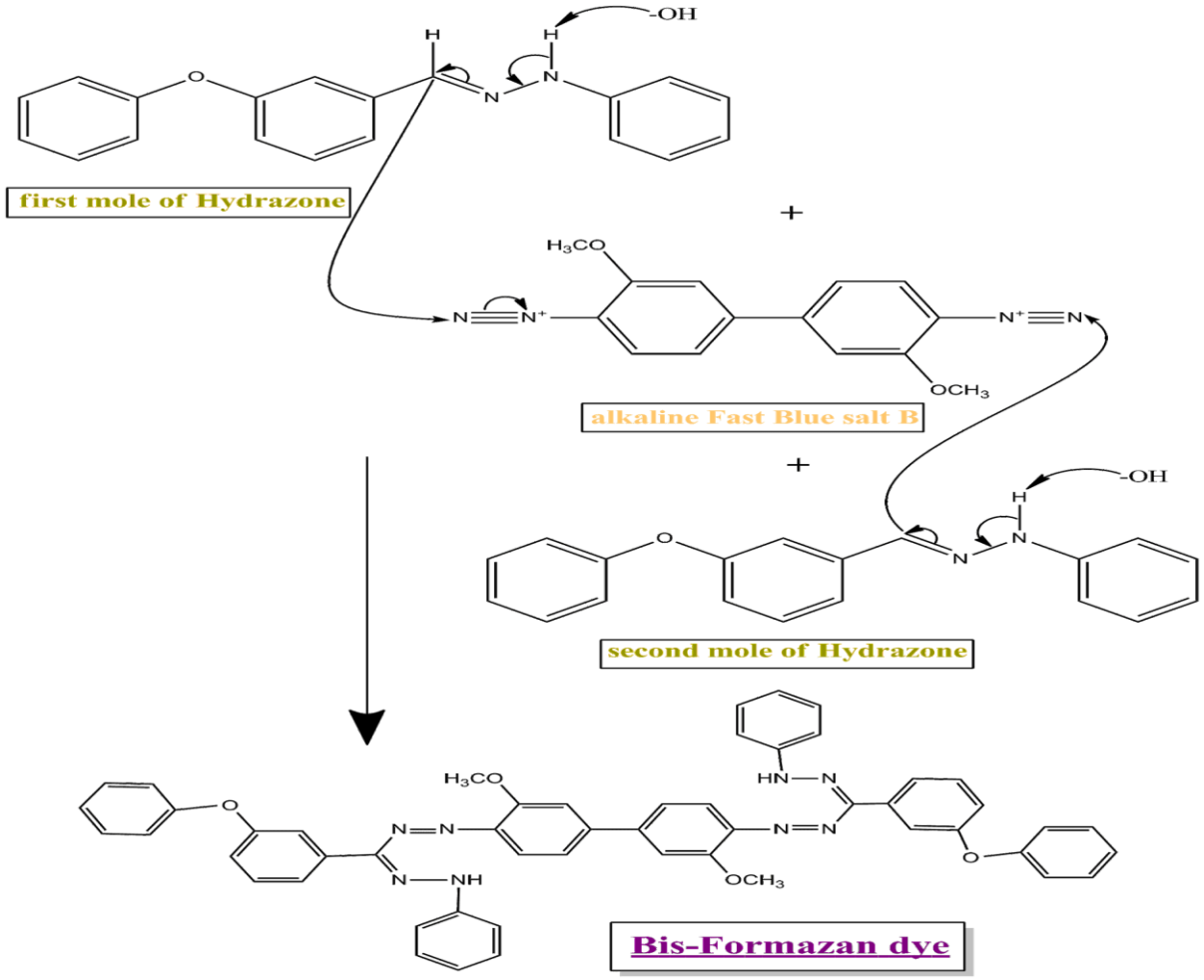

Figure-4: Formation of Formazan from hydrazones and fast blue salt-B

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The formazan thus formed had purple colour (Figure-5).

Figure-5: Spots of Formazan on TLC

If one compares the two TLC's, it can be easily seen that the spots of formazans (Figure-5) are much more intense, easy to identify and red shifted than spots of hydrazones (Figure-3). Thus spraying a diazo compound over hydrazone has increased intensity of spots, and it has also changed its colour from a less desirable yellow to more desirable purple, which is a considerable jump in spectrum. Thus this method not only detects aldehydes, but also confirms their presence over TLC plate.

4. Conclusion:

The reported method applies knowledge from dye industry to develop a thin layer chromatographic method for the detection and identification of aldehydes with synthetic pyrethroids as real samples using an ultra low cost technique. This method provides much better results than what hydrazones can offer. Aldehydes and ketones can not only be detected but their presence can be confirmed by this technique, so this can also work as a confirmatory test for aldehydes and ketones. The method was so sensitive that we had to completely skip the use of acetone as solvent in solvent system. Acetone itself formed hyrazone and then formazan dye along with the actual analyte making non-chromatographic zone also colored. The method was also found suitable for spot and wet test. Most of the synthetic chemist had reported synthesis of formazan which involve preparation of diazo compounds by diazotizing aromatic amino group under cold condition which require stringent requirement of pH, concentration and temperature and is itself a very lengthy procedure. The current method utilizes fast blue salt-B leaving behind all the labour, stringent reaction condition and most importantly lengthy work required to synthesize diazo compound. Many different variations in aryl hydrazine and diazo compounds are possible giving many different possibilities for the detection of carbonyl compounds under analysis. The reagent like fast blue is used for detection of phenols and is thus easily available in most analytical laboratories. Again, Fast blue salt-B has two diazo group giving bis-formazan which was more red shifted than mono-formazan. The false positive results which may arise by using fast blue due to presence of phenols, amines and active methylene compounds can be easily removed by spraying another plate first with only fast blue. We also want to point out an important fact regarding detection of these three types of compound using diazo reagent i.e. these three are not the only types of compounds which reacts with diazo reagent giving coloured product but hydrazones can also act as an important nucleophile which produces colour with diazo compound. Finally

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to conclude, this method provides a powerful route to detect aldehydes, ketones, hydrazine group and also hydrazones.

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