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Highly Sensitive and Specific Tandem Mass Spectrometric Flow Injection method for the Identification of Pyrethroids

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Abstract

There is a continuing need for improved or new methods for the identification of compounds of forensic interest from forensic samples. Pyrethroids, though, widely used for pest control activities, has also been reported world wide from time to time involving its poisoning. We have developed Flow injection electrospray ionization tandem mass spectrometry as a rapid and powerful technique to identify pyrethroids (i.e. cypermethrin, deltamethrin and fenvalerate).

Keywords Pyrethroids, FIA, MRM, Identification

1. Introduction

Pyrethroids are widely used in agricultural and household activities to control pests [1], and accounted for more than 25% of the worldwide insecticide market [2]. The use of pyrethroid pesticides in agriculture are rapidly increasing due to their limited toxicity to mammals and their good spectrum of activity against crop damaging pests [3]. However, adverse effects in humans may still occur following exposure to these compounds, with neurotoxicity being the primary side effect following acute exposure [4]. Deltamethrin act by delaying closure of sodium channels, resulting in a tail current that is characterized by a slow influx of sodium during the end of neuronal depolarization [5,6]. Synthetic pyrethroids have been reported to have reproductive and endocrine disrupting effect [7]. In recent past it has been observed that pyrethroids are used for homicidal purposes and also responsible for accidental poisoning. Number of techniques have been reported in the literature for the analysis of pyrethroids; like thin layer chromatography (TLC) [8,9], gas chromatography (GC) [10-15], High performance liquid chromatography (HPLC) [16,17] and Liquid chromatographic Mass Spectrometry (LC-MS) [18]. All the above methods are based on chromatography, hence analysts may face some problems like resolution, peak shift, peak tailing, longer run times and etc. Flow injection analysis coupled to Atmospheric Pressure Ionization Mass Spectrometry is likely to be a convenient technique for fast, accurate, sensitive and specific analysis. Since Flow Injection Atmospheric Ionization Mass Spectrometry has recently been shown to be an efficient technique and able to screen the analytes in complex mixtures such as bacterial identification from crude cell extracts[19], saccharides in beer samples[20], guanidinoacetate and creatine in dried blood spots[21], nifedipine[22] and topiramate[23] in human plasma, SC-68328 in dog plasma[24], thiabendazole, imazalil and o-phenylphenol in citrus fruits[25], succinylacetone in urine and dried bloodspots[26], anionic, cationic and nonionic surfactants in water[27] and polyethylene glycol 300 in drug formulation [28]. Flow injection ionspray tandem mass spectrometry has also been used for screening drugs of abuse[29]. Louden etal had build and operated a Flow Injection spectroscopic analysis system capable of providing UV, IR, 1H-NMR and Mass spectra together with atomic composition based on accurate mass determination[30]. Flow injection analysis /tandem mass spectrometry had also used in the determination of Hydrogen peroxide by adduct formation with a dinuclear iron(III) complex[31]. Therefore, it made us to develop an analytical method to identify pyrethroids via. Flow injection electrospray ionization tandem mass spectrometry (FI-ESI-MS/MS). In this study, structurally closely related pyrethroids i.e. deltamethrin, fenvalerate & cypermethrin as shown in Figure 1. are taken in to consideration and an attempt has been made to identify the product ion of highest intensity and to study its fragmentation pattern. The multiple reaction monitoring (MRM) mode had been selected to differentiate the selected pyrethroids by Flow Injection Analysis.

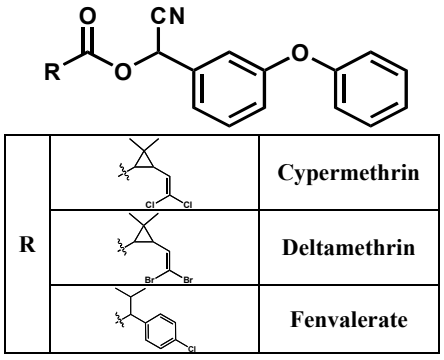

Figure 1.

2.Experimental

2.1 Chemicals

Standard pyrethroids (cypermethrin, fenvalerate and deltamethrin) were procured from Sigma-Aldrich, India. Methanol (MeOH), Acetonitrile (ACN) of HPLC grade and formic acid of analytical grade were purchased from Merck, India. Ultra pure water, obtained from a Milli-Q water purification system from Millipore (Bedford, MA, USA), was used.

2.2 Preparation of Stock

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Standard stock solution of Pyrethroids (cypermethrin, fenvalerate and deltamethrin) (1mg/ml) were prepared by exactly weighing and dissolving in methanol, protected against light and stored at 4^0C in a freezer. Working standard solutions with concentrations of 1.0, 2.0, 10.0, 20.0, 40.0, 100.0, 200.0, 600.0 and 1000.0 ng/ml were prepared by appropriate dilution of the 1.0 mg/ml stock solution with methanol, there after 5.0 ml of each working standard had made up to 10.0 ml with 0.2% formic acid aqueous solution; as a result the final concentrations were 0.5, 1.0, 5.0, 10.0, 20.0, 50.0, 100.0, 300.0 and 500.0 ng/ml respectively.

2.3. Instrumentation and data processing

FI-ESI-MS/MS was conducted on a Perkin Elmer (USA) Series 200 system (consisting of a vacuum membrane degasser, a gradient pump and an auto sampler) coupled to the mass spectrometer, Applied Biosystems MDS Sciex (Canada) API3200 Q Trap Triple Quadrapole mass spectrometer using electro spray ionization (ESI) interface, in the positive-ion mode. The data acquisition and data processing was done by Analyst 1.4.1. software supplied by Applied Biosystems MDS Sciex (Canada). The solvent system was of formic acid 0.1% in methanol–water (75:25) at a flow-rate of 200µl/min and the sample injection volume was 20 µl.

3. Results and Discussion

The optimization of the MS conditions was carried out by direct infusion of a standard solution containing 500ng/mL of each pyrethroid into the MS/MS. Mass spectrometer cycle time (Dwell Time) were optimized to ensure a minimum of 12 data points across each peak. A set of Source Parameters were optimized commonly for all the pyrethroids as shown in the Table 1.

Table 1. Source Parameters

Parameter	Optimum Valve
Ion Spray Voltage	4500v
Nebulizer Gas	25 psi.
Auxiliary Gas	40 psi.
Heater Temperature	200 ^{0}C
Curtain Gas	20 psi.
CAD Gas	6 psi.
Dwell Time	200 m.sec.

For every pesticide the MS signal was optimized and the maximum single for the pseudo molecular ion $[M+H]^+$ was observed in positive ionization mode for all compounds. On the other hand, the addition of additives solutions was evaluated and the results obtained showed that the MS signals increased when formic acid was added to standard solutions for all target analytes. Thus, a concentration of 0.1% of formic acid was selected in order to increase the sensitivity of the pyrethroid signal. There after the selected pseudo molecular Ion was fragmented via. collision activated dissociation (CAD) in the collision cell by applying collision energy and 99.9% N_2 gas as CAD gas in the product ion scan and the most abundant fragment is identified. Finally in MRM mode pseudo molecular ion is first selected, the selected pseudo molecular ion is fragmented in collision cell and then fragment of specific mass in order of response is detected. This MRM scan type is very specific for target compound analysis. The exact mass, *m/z* of pseudo molecular ion and the most abundant fragment of the selected pseudo molecular ion are tabulated in Table 2.

The FIA method was optimized in order to evaluate the influence of mobile phase composition on the ionization efficiency. Different mobile phases using aqueous binary mixtures of formic acid with organic solvent (MeOH or Acetonitrile) were experimented. The best MS signals were obtained with MeOH as described in the 'Instrumentation' section. The MRM mode is selected for identification of pyrethroids using the peak height of the MRM transitions of each compound. Limit of detection (LOD) of pyrethroids are found to be as cypermethrin 0.5ng/ml; deltamethrin 1ng/ml and fenvalerate 1ng/ml taking into consideration that signal to noise ration is 3. Cumulative peaks of the three pyrethroids and the separate peaks of the three pyrethroids have been shown in Figures 2-3.

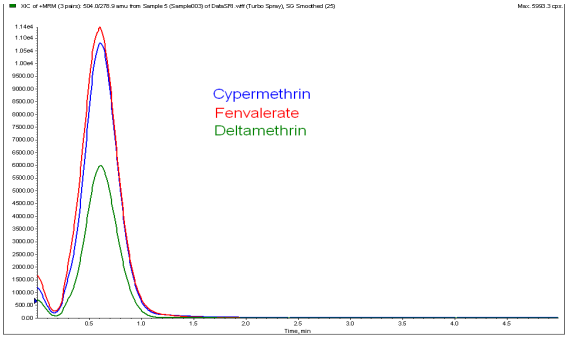
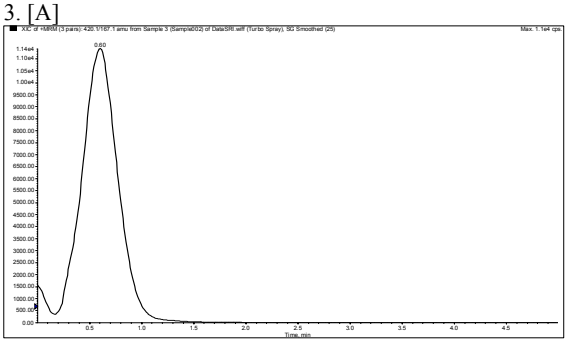


Figure 2. Cumulative peaks of the three pyrethroids of 100ng/ml

3. [A]



3. [B]

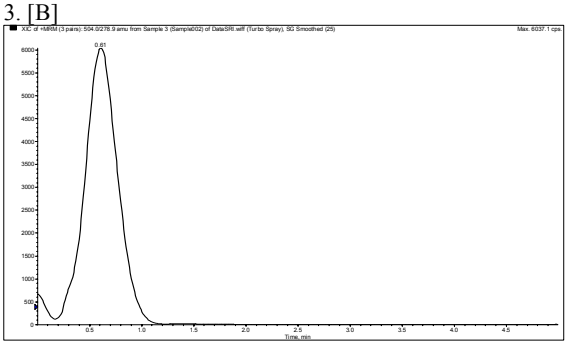


Table 2. Compound Parameters

			For Multiple Reactions Monitoring (MRM)			
Pesticide	Molecular Formulae	Exact Mass (g/mol)	Pseudo Molecular ion (*m/z*)	Product ion (*m/z*)	Declustring Potential (v)	Collision Energy (v)
Cypermethrin	$C_{22}H_{19}Cl_2NO_3$	415.0742	416.0820	191.0030	34	15
Deltamethrin	$C_{22}H_{19}Br_2NO_3$	502.9732	503.9810	278.9020	30	15
Fenvalerate	$C_{25}H_{22}ClNO_3$	419.1288	420.1366	167.0628	29	18

3. [C]

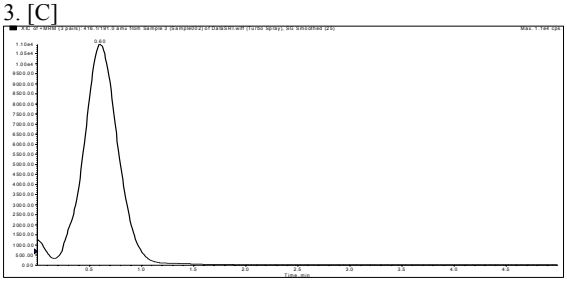
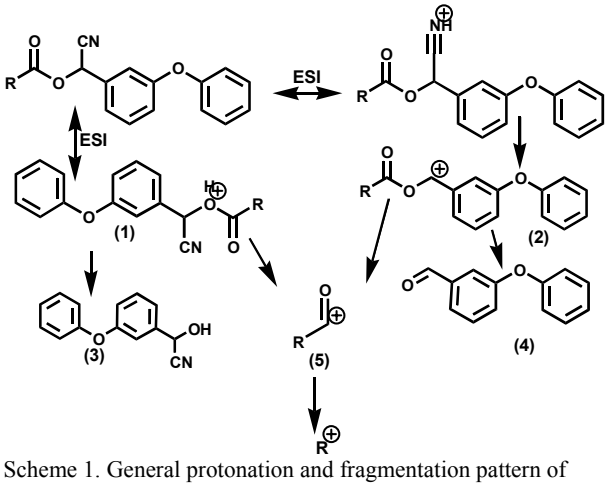


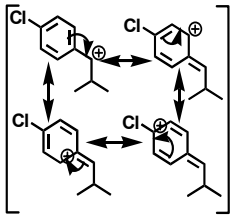
Figure 3. [A, B and C] Separate peaks of fenvalerate, deltamethrin and cypermethrin respectively.

In all the three compounds ionization was found to be similar at first stage i.e. protonation occurred on the ester group or on the nitrile group and there after the molecule has fragmented as shown in Scheme 1. The fragmentation pattern included liberation of hydrogen isocyanide, there after the formed ion (marked as 2) knocked out a neutral molecule 3-Phenoxy-benzaldehyde (marked as 4) and produced the ion (marked as 5). The other possible path way was that the ion (marked as 1) gets hydrolysed to give ion (marked as 5) and neutral molecule Hydroxy-(3-phenoxy-phenyl)-acetonitrile (marked as 3). There after ion (marked as 5) gives out carbon monoxide.



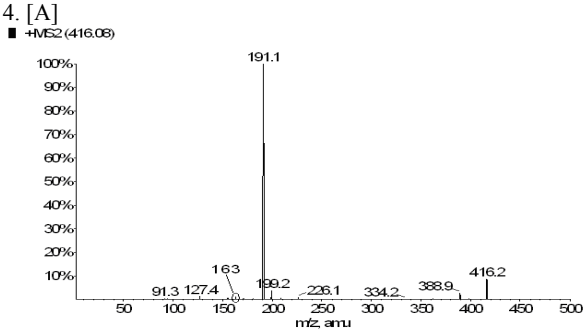
Scheme 1. General protonation and fragmentation pattern of pyrethroids

Protonated deltamethrin hydrolysed to give product ions at *m/z* 278.9020 $(C_8H_9Br_2O)^+$ and protonated cypermethrin hydrolysed to give product ions at *m/z* 191.0030 $(C_8H_9Cl_2O)^+$ as intense product ions. However the process is some what different in case of Fenvalerate. The most intense product ion was observed at *m/z* 167.0628 $(C_{10}H_{12}Cl)^+$ instead of *m/z* 195 $(C_{11}H_{12}ClO)^+$. This characteristic behavior may be attributed to the resonance stabilization of the product ion *m/z* 167.0628 after liberation of carbon monoxide as shown in scheme 2. The product ion spectrum of cypermethrin and fenvalerate are shown in figure 4. The other common fragment in all the three selected pyrethroids was ion (marked at 2). In case of cypermethrin the *m/z* of this ion was at 389, in deltamethrin it was 477 and in case of fenvalerate it was 393.



Scheme 2. Resonance Structures of *m/z* 167

4. [A]



4. [B]

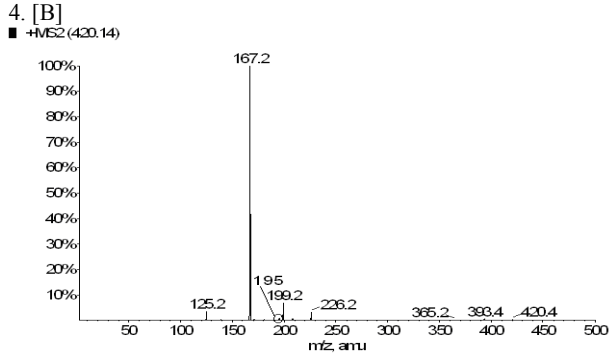


Figure 4. [A and B] Product ion mass spectrum of cypermethrin and fenvalerate respectively

4. Conclusion

This study has demonstrated the power of FI-ESI-MS/MS as a simple, rapid and efficient method. The demonstrated novel mass spectrometric method is the most rapid of any published mass spectrometric methods. FI-ESI-MS/MS has a potential as an analytical tool for the identification of pyrethroids. MRM provides a sensitive and specific means for analyzing concentrations at LOD of level 0.5ng/ml for cypermethrin, 1ng/ml for deltamethrin and 1ng/ml for fenvalerate. The possible explanations of structure elucidation of various fragments formed in product ion spectrums of cypermethrin and deltamethrin including the resonance behavior of fenvalerate enables to differentiate these structurally closely related compounds.

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