GC/CI-MS/MS method for the identification and quantification of N-nitrosoethylmethylallyl-amine in plant protection products

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Abstract A simple and sensitive method for trace level determination of the n-nitrosamine, N-nitrosoethylmethylallyl-amine in emulsifiable concentrate pesticide formulations is described. Solid phase extraction is used as a clean-up step of the sample before chromatographic analysis. Instrumental analysis involved gas chromatographic separation coupled to tandem mass spectrometry using Positive Chemical Ionization with methane as the reagent gas. (GC-CI-MS/MS). A gas chromatographic method with flame ionization detection (GC-FID) was used supplementary when the concentration levels of the analyte were higher than 50 μg g^{-1}. Both methods were validated with respect to linearity, accuracy, limit of detection and quantification as well as specificity. The average recoveries of the two fortification levels varied from 96.4 % to 98.5 % and the RSDs ranged between 2.3 % and 7.4 %.

Keywords: dinitroaniline herbicides, n-nitrosamines, N-nitrosoethylmethylallyl-amine, plant protection products

1. Introduction

N-Nitrosamines are a chemical class of organic compounds that are exceptionally toxic to animals and are considered potential human carcinogens and mutagens. N-nitrosamines are readily formed by the reaction between primary or secondary amines and nitrosating agents such as nitrous acid, nitrites or nitrogen oxide. Nitrosamines have been detected in different matrices e.g. environmental samples such as air and water, in food, cosmetics, tobaco etc. The list of products that have been demonstrated to contain N-nitroso compounds has grown considerably over the past decade. Dinitroaniline herbicides, a group of nitro aromatic compounds, are a class of highly efficient pre-emergence herbicide widely used to control a variety of annual grasses and certain annual broadleaf weeds in cotton, soybean and corn. They have been found to contain N-nitrosamines and for that reason a specification of a maximum content of 1mg kg^{-1} in the technical grade active ingredient (TGAI) has been set in SANCO/10597 EU guidance document.

The most commonly used dinitroaniline herbicides include ethalfluralin (ETL), trifluralin (TFL) and pendimethalin (PTL). A well-known issue during the production at industrial scale of dinitroanilines' TGAI is the formation of N-nitroso by-products. In order to achieve the reduction of their concentration at acceptable levels an extra clean-up step is required. However, this extra step constitutes a significant increase to the cost of the final product. Due to free-market competition and the need for reduction of costs this step seems to be omitted by some manufacturers. Thus it is not unusual during market controls to find dinitroaniline herbicide products containing high concentrations of N-nitrosamines. Ethalfluralin being a dinitroaniline compound also falls under the requirements of SANCO/10597 EU guidance document for monitoring nitrosamines (c< 1mg kg^{-1} in TGAI). The development and validation of accurate and sensitive methods for the determination of n-nitrosamines in dinitroaniline herbicide plant protection products is of high importance. Many methods that have already been published in the international literature are based on a variety of techniques such as chromatographic, spectroscopic and electrochemical techniques. The most suitable and widely used methods for the determination of nitrosamines are gas chromatography coupled with different detectors such as nitrogen-phosphorous, mass spectrometry and thermal energy analyzer.

This work sought to develop a fast and simple gas chromatographic method coupled to positive chemical ionization tandem mass spectrometry (GC-CI-MS/MS) for the determination of N-nitrosoethylmethylallyl-amine (EMANA) in Emulsifiable Conncentrate (EC) plant protection products containing ethalfluralin as an active ingredient. The GC-CI-MS/MS method was developed in order to achieve a limit of quantification lower than SANCO specification limits (1mg kg^{-1} in TGAI). However, in many samples the concentration levels of the analyte (EMANA) were found to be at exceptionally high levels and thus GC with flame ionization detection (GC-FID) was applied in order to avoid contamination of the MS/MS system and to take advantage of the FID detector robustness. The method thus developed for both techniques was validated for specificity, linearity, precision and accuracy. The greatest advantage of the proposed method is the highly specific determination of the analyte (EMANA) in the very complex and highly concentrated

(33% w/v in ethalfluralin) matrix of the EC plant protection product.

2. Experimental

2.1. Reagents and materials

Analytical standard of N-nitrosoethylmethylallyl-amine (99%) was donated by DowAgrosciences (Norfolk, UK). HPLC-grade methanol, acetone, 1-chlorobutane and hexane were obtained from Fischer Scientific (Fisher Scientific, USA). Stock and working standard solutions for method validation were prepared in acetone in the range of 0.1 to 1 µg ml^{-1} for GC-MS/MS analysis and of 2.5-110 µg ml^{-1} for GC-FID analysis.

2.2. Sample preparation

It should be noted that due to the sample complexity and the low concentration of the target analyte (EMANA) in most of the studied PPPs there was an increased difficulty in the clean-up and sample preparation step. Solid Phase Extraction (SPE) (ISOLUTE®, Biotage, Uppsala, Sweden) using Silica gel cartridges was applied in order to remove the high concentration of ethalfluralin (a.i.) and co-formulants thus obtaining an enriched sample in the analyte (EMANA). The procedure was as follows: The silica bed was wetted with methanol and hexane. On the upper part of the cartridge a layer of basic alumina and dry sodium sulfate was placed. The bed was then air dried under vacuum and then wetted with 1-chlrorobutane. One (1) gram of the EC PPP sample was then introduced in the cartridge bed, washed with 1-chlorobutane and air-dried. The basic alumina and dry sodium sulfate layer together with the cartridge frit were removed and the silica was transferred to a vial. A mixture of 1-chlorobutane: methanol (90:10) was then added and the resulting slurry was hand-shaken and finally filtered through a 0.45µm filter in an amber sample vial. The solution was evaporated under a gentle stream of nitrogen until volume of 1 ml.

2.3. GC-MS/MS analysis

GC-MS/MS analysis was performed on a Varian CP-3800 gas chromatograph, with electronic flow control and was interfaced to a 1200L mass selective triple quadrupole mass spectrometer system (Varian, Palo Alto, California USA). Chromatographic separation was achieved using a VF-1 MS (30m × 0.25mm × 0.25µm film thickness) capillary column along with a programmable temperature vaporization (PTV-1079) injector operating at the split mode with split ratio of 100 and held at a temperature of 250^{o}C. The GC oven temperature was maintained at 50°C for 1 min, and then ramped to 135°C at a rate of 10^{o}C min-1 for 9 minutes and final ramped to 280^{o}C at a rate of 10^{o}C min-1 for 30 minutes. The flow rate set at 0.5 ml min^{-1} and the injection volume at 1 µL ml^{-1}. The autosampler used was a CTC Combi-Pal (Switzerland). Mass spectrometry was performed in positive chemical ionization mode (CI) at 70eV ionization energy. Methane was used as reagent gas at 9.5m Torr. For the MS/MS experiments argon was used as a collision gas and the collision cell pressure set at 1.5 m Torr. The temperatures of the ion source, transfer line and manifold were set at 250^{o}C, 280°C and 40^{o}C respectively. The electron multiplier set at 1300V. Multiple reaction monitoring (MRM) conditions were experimentally developed for the analyte (EMANA) used in this method by selection of the most abundant fragments from the full scan spectrum (Figure 1).

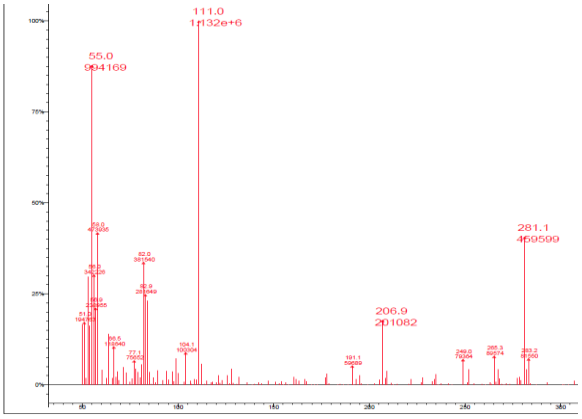

Figure 1. GC-MS full scan spectrum of standard solution 0.4 ug/ml of EMANA

The relevant considerations included the choice of precursor ion, product ions and optimization of collision energies for best response. Collision energy, precursor and product ions were m/z 129>55 (qualification ion) (collision energy 8 V), 129>70 (qualification ion) (collision energy 9 V), 129>84 (quantification ion) (collision energy 7.5 V), 129>99(qualification ion) (collision energy 4 V). The analyte in plant protection products analyzed from the market were identified on the basis of their retention time as well as at least two transitions. Instrument control and results' processing were carried out using MS Workstation version 6.8.

2.4 GC-FID analysis

Chromatographic analysis for the quantitation of the nitrosamine under study was carried out on a ThermoFinnigan Trace GC (Thermo Fisher Scientific Waltham, MA USA) equipped with a split/ splitless injector, operated in splitless mode, a Flame Ionization Detector and an autosampler (ThermoFinnigan AS 2000). The chromatographic column used was a DB-1 (30m x 0.53 mm, 1.5µm film thickness, J&W Scientific, Folsom, California). Instrument control and results' processing were carried out using ChromCard software (Thermo Fisher Scientific Waltham, MA USA). The chromatographic conditions were: helium as a carrier gas set at pressure 45 kPa, both detector and injector temperatures set at 250°C, injection volume 1 mL. The GC oven temperature was maintained at 60°C for 5 min, and then ramped to 180°C at a rate of 5^{o}C min^{-1} and final ramped to 270^{o}C at a rate of 10^{o}C min^{-1} for 20 minutes.

3. Results and Discussion

3.1 Method validation

The proposed analytical method used for the determination of n-nitrosamine EMANA in ethalfluarlin pesticide EC formulation samples was validated regarding linearity, limit of detection (LOD) and quantification (LOQ). Linear range was established with a series of working solutions as described above at a range of 2.5-110 μg ml^{-1} with a correlation coefficient of R^2=0.999 for GC-FID analysis and 0.01 to 1 μg ml^{-1} with a correlation coefficient of R^2=0.997 for GC-(CI)-MS-MS analysis. Repeatability of five replicate injections of standard solution gave an RSD of 0.21% in the case of GC-FID and 1.2% in GC-MS-MS. The accuracy of the proposed method was determined from recovery experiments at two spiking levels through standard amounts of the analyte in well-characterised samples in both cases. Recoveries ranged from 96.4 to 98.5% in both cases with RSD % ranged from 2.3 to 7.4%. The LOQ of the method was defined as the lowest recovery provided acceptable RSD. In the case of GC-FID the LOQ was 20 μg g^{-1} whereas for GC-MS-MS the LOQ defined as 0.01 μg g^{-1}. The above results exhibited the reliability and accuracy of the measurement of EMANA in commercial pesticide formulations, indicating an absence of systematic error in the developed method. The chromatographic peak of EMANA was identified according to molecular masses obtained by GC-(CI)-MS-MS in the positive-ionisation mode.

3.2 Analysis of commercially available samples

In order to confirm the viability of the proposed method, the method was successfully applied to determine the concentrations of EMANA in real samples (1a-1e) from the Greek Market. It should be noted that according to European Guidance documents (SANCO/10597) n-nitrosamines should be at concentration levels lower than 1 μg g^{-1} in the technical grade active ingredient which corresponds to 0.33 μg g^{-1} in the EC 33% w/v products of the Greek market. The results presented in Table 1.

Table 1. Concentration of EMANA in samples from the Greek market

Samples No	Concentration of EMANA in the PPP μg g^{-1}	Concentration of EMANA in the technical material (TGAI) μg g^{-1}
1a	67.7	203.1
1b	72.2	216.6
1c	71.3	213.9
1d	<LOQ	<1 (specified limit)
1e	<LOQ	<1 (specified limit)

Sample with sample No 1a, 1b, 1c were analyzed with GC-FID whereas samples with sample No 1d, 1e were analysed with GC-MS-MS (Figure 2). From the results obtained it can be concluded that some samples of PPPs from the Greek market containing ethalfluralin as active ingredient had unacceptable levels of EMANA according to EU tolerance limits.

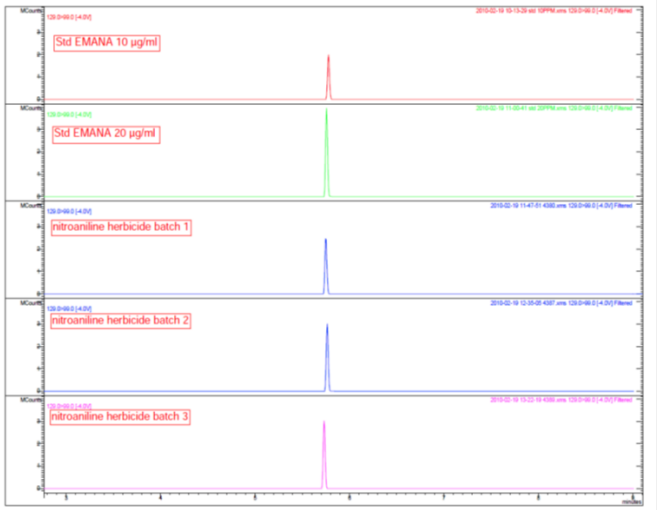

Figure 2. GC-CI-MS/MS chromatograms of standard solutions of EMANA and formulation sample solutions.

4. Conclusions

Quality control of plant protection products is an important and composite task. Market control of PPPs must address the crucial issue of TGAIs containing relevant impurities of toxicological concern in order to assure the protection of the health of PPP end-users, consumers, and the environment.

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