

Determination of amines using HPLC with fluorescence detection

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Abstract:

A simple and sensitive method for the determination of free aliphatic and aromatic amines using derivatives agents (dansyl chloride, 9-fluorenylmethyl chloroformate and 4-Chloro-7-nitrobenzofurazan) as a labelling reagent by high-performance liquid chromatography with fluorescence detection (HPLC-FLD) has been developed. Derivatization conditions including reagent concentration, buffer pH, reaction time and temperature were optimized. A fluorescence derivatization - high-performance liquid chromatography (HPLC) method, which enables the femtomole-level detection of analytes, is a powerful tool for the analysis with high sensitivity and selectivity.

In recent decades, improvised explosive devices (IEDs) have become an increasing topic of public concern, with high-profile incidents such as the Bali (2002), London transport (2005), Boston Marathon (2013) and Brussels (2016) bombings garnering mass international attention.[1] Although the majority of media coverage has focused on large-scale incidents, there has also been a rise in explosive incidents involving smaller devices such as pipe bombs. This is particularly the case in the United States, where pipe bombs are considered to make up the vast majority of IED encounter. These devices can be easily constructed from everyday materials.[2]

The mixture nitromethane with amines is applicable for this. Determination of residues after an explosion is important. This method (HPLC-FLD) could be useful for their determination. The proposed method is sensitive and reproducible for the determination of aliphatic and aromatic amines.

Keywords: amines; HPLC; derivatization

1 Introduction

The use of amines for making improvised explosive devices (IED) can be used in criminal practice. Amines can be used as initiator in explosive mixtures along with nitromethane. The identification of the amines used in homemade explosives can provide additional forensic information of this evidence.

A very sensitive method for determining amines is HPLC with a fluorescence detector. Unfortunately, most amines do not show fluorescence. It is necessary to carry out derivatization before the analysis. The derivatising reagent will enhance the detection of amines by UV absorbance or fluorescence. A range of pre- and post-column derivatisation reagents have been described in the literature [3-6].

2 Experimental

The HPLC system consisted of two pumps (Model LC-10ADvp) programmed with a microprocessor controller (ModelSCL-10Avp), RF-20Axs fluorescence detector (excitation at 340 nm, emission at 530 nm) and an autosampler (Model SIL-10 ADvp) all from Shimadzu (Japan). The column was KINETEX EVO - C18 (150 mm × 4,6 mm, 2,6 μm) supplied by Merck protected by a guard column (40 x 3 mm I.D.) containing a silica-based C18 sorbent packing. Dansylated amines injected onto the column were eluted with acetonitrile and water both of which had been previously passed through a 0.45 μm filter. The gradient used in the HPLC method developed in this study is follows: the initial conditions were 30% acetonitrile and 30% water pumping at a flowrate of 1.5 ml min⁻¹. For details of operating conditions see text. Acetonitrile and 30% water pumping at a flowrate of 1.5 ml min⁻¹. This mixture was pumped for 2 min, and then the concentration of acetonitrile was raised to 80% (4 min). This concentration of acetonitrile was kept constant for 4 min, and then returned to the initial conditions. Acetonitrile, dansyl chloride and amines (analytical grade, Sigma-Aldrich, Germany) were used.

The amines were derivatised according to the method of Moneeb [7] with some modifications, as follows: 400 μl of the amines were derivatised. Then, 400 μl of a 0.5 mol L⁻¹ solution of NaHCO₃ (pH = 9) and dansyl chloride (DNS-Cl, 2e-3 mol L⁻¹) were added. The resulting mixture was either incubated in the dark at various temperatures. Finally, the sample was passed through a 0.45-μm pore size syringe filter and 20 μl was injected onto the HPLC system.

Standard solution of dimethylamine was prepared in acetonitrile. The concentration level was 400 mg/l. Solutions for calibration were prepared from this solution (from 5e-7 to 1e-4 mol L⁻¹).

3 Results and discussion

To optimize the derivatization of amines, the effects of reaction time and temperature and initial DNS-Cl concentration on the derivatization performance were studied for dimethylamine, chosen as a model compound.

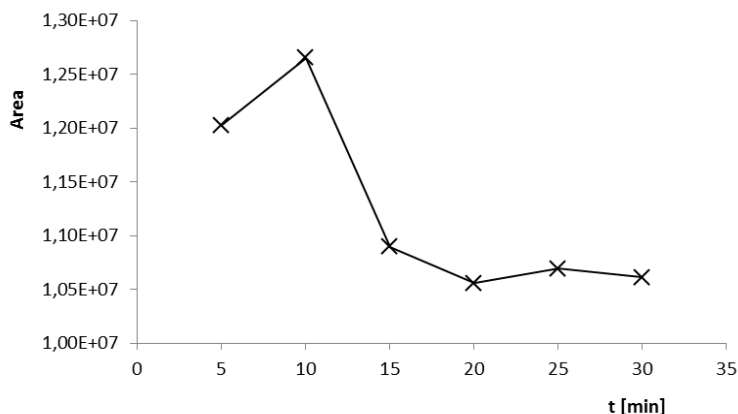


Figure 1: Effect of reaction time on the derivatization of 50 mg/L dimethylamine

A solution of 50 mg/L dimethylamine was derivatised with DNB-Cl at six reaction times ranging from 5 to 30 min and subsequently injected into the HPLC with FL detector, measuring the peak area obtained for each trial. As shown in Figure 1, the response increases with

reaction time up to 10 min, then response decreases by approximately 20%. The 10 min reaction time was chosen as optimal. Detector response is almost the same at higher times.

Once identified the optimal reaction time, volume of DNS-Cl necessary to obtain the highest derivatization yields (signal responses) was studied. The volume of the derivatizing agent (100–800 μl) was tested in the following experiments.

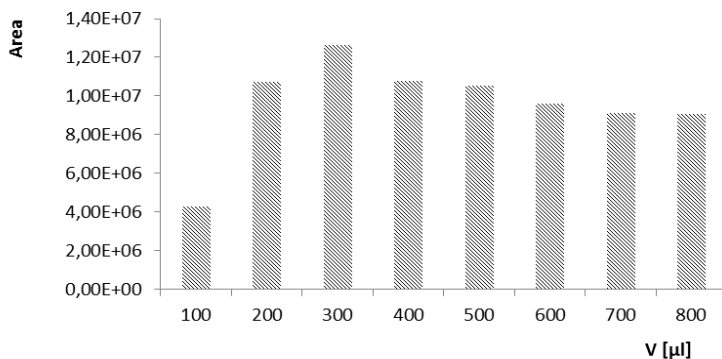


Figure 2: Effect of volume of derivatizing agent on the analytical signal of dansylated dimethylamine (50 mg/L)

The effect of volume is shown in Figure 2. The detector response is increasing to volume 300 μl , and then begins to decrease. The volume of 300 μl was chosen as optimal.

Generally the reaction kinetics depends on the reaction temperature, and this variable was studied in the range of 40–80 °C in steps of 10 °C. As can be seen in Figure 3, the analytical signal is increasing with increasing temperature. The response of the detector is almost the same at temperatures of 70 and 80 °C.

The optimal conditions for derivation are as follows: 70 °C for 10 min with a volume of derivatizing agent 300 μl . Derivatization of amine mixtures (ethylenediamine EDA-DNS, diethanolamine DETA-DNS, morpholine MORF-DNS, iso-propylamine iPROP-DNS, propylamine PROP-DNS, terc-butylamine tec-BUT-DNS, sec-butylamine sec-BUT-DNS, iso-butylamine iBUT-DNS, butylamine BUT-DNS, diethylamine DEA-DNS and cyclohexylamine CYK-DNS) was carried out under these conditions. The chromatogram is shown in figure 4. The separation of EDA-DNA and DETA-DNS is not very good, but in real samples is not mixture of amines.

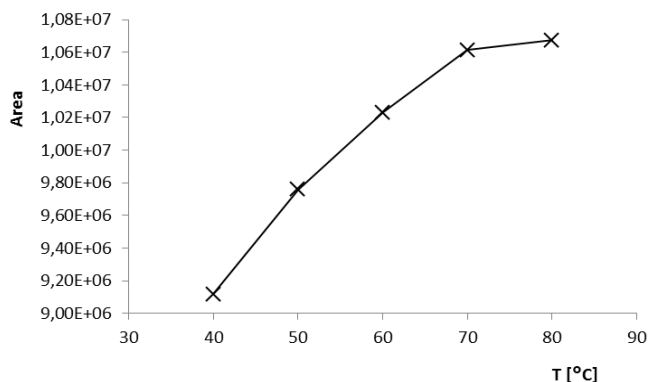


Figure 3: Effect of the reaction temperature on the analytical signal (peak area) of dansylated dimethylamine

Real samples were obtained after the explosion of a mixture of nitromethane and amine (95:5 V/V). The collection was performed with a glass swab from sheet target. Extraction of the substances was carried out in acetonitrile. The solution thus obtained was subjected to derivatization according to the conditions described above. This method has been identified diethylamine, diethanolamine, propylamine, iso-propylamine, sec-butylamine, iso-butylamine and cyclohexylamine after the explosion. The rest of the amines could not be identified after the explosion.

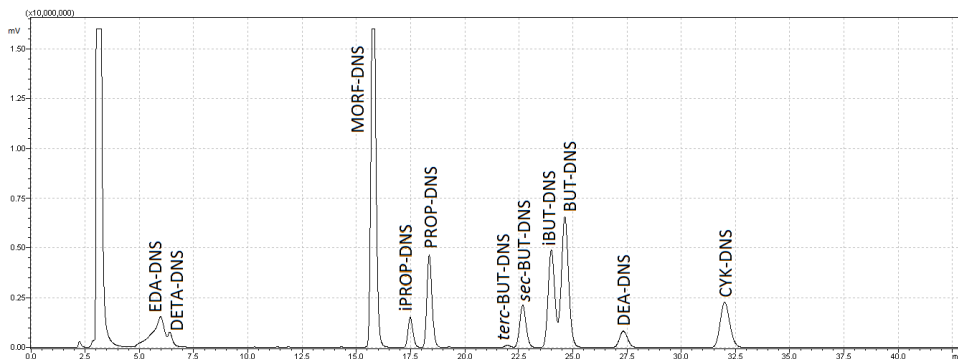


Figure 4: Chromatogram of dansylated amines (ethylendiamine EDA-DNS, diethanolamine DETA-DNS, morpholine MORF-DNS, iso-propylamine iPROP-DNS, propylamine PROP-DNS, terc-butylamine tec-BUT-DNS, sec-butylamine sec-BUT-DNS, iso-butylamine iBUT-DNS, butylamine BUT-DNS, diethylamine DEA-DNS and cyclohexylamine CYK-DNS)

4 Conclusion

In this work, suitable conditions for the determination of amines after explosion were searched. Analytical method was chosen by HPLC with a fluorescence detector. Its use requires the derivatization of amines. Dansyl chloride was chosen as the derivatizing agent. Results presented in this work show that this analytical method is sensitivity for their determination after explosion.

Acknowledgments

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