Determination of Methylamine, Dimethylamine, and Trimethylamine in Air by High-Performance Liquid Chromatography with Derivatization by 9-Fluorenylmethylchloroformate

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Abstract

An HPLC-UV method coupled with 9-fluorenylmethylchloroformate (FMOC) derivatization was developed for the determination of short chained amines in an environmental matrix. The basic reaction conditions among different target compounds (three methylated amines: mono- (MA), di- (DMA), and tri- methylamine (TMA)) with reagent (FMOC) have been investigated. Comparative calibration of TMA as individual target and in a mixture (i.e., with MA and DMA) indicated enhanced sensitivity of the former (response factor (RF) of 7593) and a suppressed pattern for the latter (RF=3732). According to the kinetics studies, the minimum of 40 min was required for their derivatization. The detection limits of MA, DMA, and TMA derived using liquid standards were 0.12, 0.08, and 0.05 ng, respectively.

To validate the applicability of this method, an environmental sample was analyzed by derivatizing amines released from rotten fish. For this purpose, a simple impinger method based on dynamic headspace sampling was developed to collect amine gas. For derivatization, gas sample was passed through a train of three impingers (with FMOC in acetonitrile solution). The analysis of real sample made using a rotten fish (thornback ray*: Raja clavata*) yielded significantly high concentrations of MA (61 ppm) and TMA (190 ppm) with their overall capture and derivatization efficiencies of 93 and 98%, respectively. Its spoilage level, evaluated in terms of the total volatile basic nitrogen (TVBN), corresponded to 38.2 mg N/100 g of fish, confirming biodegradation of fish as the potent source of amine.

Keywords: Amines, FMOC, derivatization, kinetics, total volatile basic nitrogen (TVBN), dynamic headspace

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Introduction

Short chained aliphatic amines (e.g., methylamine (MA), dimethylamine (DMA), and trimethylamine (TMA)) are well known for their potential in the formation of secondary organic aerosols.[1, 2] Moreover, they are widely publicized malodorants (pungent, rotten-fish like smell) with low odor threshold values (in a range of 21-35, 33-47, and 0.032-0.21 ppb (v/v), respectively for MA, DMA, and TMA).[3] Amines in the presence of nitrogen oxides or other nitrosating agents can easily form N-nitrosamines which can pose potential health hazards as mutagens and carcinogens.[4, 5] Their health effects also include irritation of eyes, skin, and upper respiratory tract, coughing, difficulty of breathing, lung edema, etc.[6-8] Considering the widespread use of amines in different industries (e.g., manufacturing, agriculture, pharmaceuticals, paper, rubber, petroleum, carbon dioxide capture, etc.) a special concern is required to limit their atmospheric emissions.[9, 10]

In the analysis of short chained aliphatic amines in environmental matrices, gas chromatography (GC) or liquid chromatography (LC) has been the common choices for the analysis.[11, 12] However, the basic polar amines are not suitable for GC analysis as they are strongly retained by the silanol groups and siloxane bridges on the stationary phase of the GC capillary column leading to excessive retention times and poor peak shapes.[9] From this perspective, HPLC with UV detection can be a preferable option. On the other hand, simple aliphatic amines lack suitable UV chromophores for UV detection. As a result, derivatization with reagents possessing suitable UV chromophores may be considered one promising option to facilitate sensitive detection of amines. [13-18]

Sampling and/or pretreatment technique is another important issue to accurately measure environmental samples.[9] In general, sampling techniques employed for amine analysis include solid phase extraction (SPE) [19, 20], solid-phase microextraction (SPME) [9], liquid phase microextraction (LPME) [21], and liquid-liquid extraction (LLE). [22] For the sampling of airborne amines, the commonly used methods include sorbent tubes (STs), cartridges (e.g., C_{18} cartridges), annular-denuders, and midget impingers. [9, 20, 23-25]

In this study, a number of experiments were conducted to analyze gaseous amines through the combination of chemical derivatization and HPLC-UV analysis. MA, DMA, and TMA were selected as target considering their wide environmental distribution and similar odor properties. [7, 26] 9-fluorenylmethylchloroformate (FMOC) was chosen as derivatization reagent for its unique ability to derivatize primary (MA), secondary (DMA), and tertiary (TMA) amines simultaneously. [23, 27-30] Moreover, the derivative products of these amine-FMOC derivatization reactions (e.g., FMOC-carbamate (MA and DMA) and acyl ammonium salt (TMA)) should retain suitable chromophoric properties. [25]

In this study, experiments were done in two different stages. In the first stage, five types of calibration experiments (**Exp 1 through 5)** were conducted to optimize the amine-FMOC derivatization conditions: **(1)** initial testing of solvent (acetonitrile) and reagent (FMOC) for trace impurities and ghost peaks, **(2)** determination of FMOC-TMA derivatization reaction time, **(3 and 4)** derivatization optimization in both individual and mixture amine standards, and **(5)** estimation of optimal FMOC/amine ratio for derivatization. The developed methodology was then successfully applied to real samples with the aim of quantifying amines (second stage). As our proposed method is simple and readily applicable to relatively unsophisticated instrumentation, it can thus be easily applied for the analysis of amines in real gas phase samples.

Materials and methods

Apparatus and reagents

For the analysis of all three amines, an HPLC system (Lab Alliance 500) consisting of a preparative pump, a 20 μL sample loop injector, and an ultraviolet–visible spectroscopy (UV-Vis) detector operating at 362 nm was employed **(Table 1A)**. After injection of the derivatized amine samples, the three different amine derivatives were separated by a Hichrom 5 C_{18} analytical column (HI-5C18-250A; column dimension: 250 mm (*l*) × 4.6 mm (*id*); particle size-5 μm). A 7:3 volumetric mixture of acetonitrile and distilled water was used as mobile phase for optimal separation based on our previous work. [31, 32] UV detection of each amine derivative was made at 262 nm.

The raw chemicals of all three amines (as aqueous solutions: 40% for MA and DMA, and 25% for TMA) and reagent FMOC (99% purity) were purchased from Sigma-Aldrich, Inc., USA (**Table 1B**). HPLC ultrapure grade (99.99%) acetonitrile was purchased from J.T. Baker (USA).

Preparation of amine-FMOC standards for calibration purposes

Primary standards (PS) of MA, DMA, and TMA were prepared independently in three different vials by adding 4.4, 6.3, and 12.8 μL of MA, DMA, and TMA aqueous solutions, respectively with acetonitrile to make a 5 mL solution (concentrations corresponding to 10029, 9969, and 10030 pmol/μL, respectively). Those primary standards were used to prepare different working standards (WSs). To prepare the primary standard of FMOC (PS-F) at 0.01 M, 0.0125 g of FMOC powder was dissolved with acetonitrile to make a 5 mL solution (at 25°C).

For **Exp 1**, WS of FMOC alone were prepared at ten different concentration levels (5.15, 10.3, 15.5, 30.9, 51.5, 103, 206, 412, 824, and 1546 pmol/μL)**.** In **Exp 2**, WSs of TMA prepared at two different concentrations (A. 25 and B. 12.5 pmol/μL) were derivatized with FMOC of 25 pmol/μL level. In **Exps 3 and 4,** WSs of amines (MA, DMA, and TMA) were prepared (both individually and as a mixture) at eight different concentration levels, while a molar excess of FMOC was used for derivatization (**Table 2**). In **Exp. 5**, WSs of TMA prepared at five different concentrations (5.00, 10.0, 20.0, 40.0, and 75.0 pmol/μL) were derivatized at varying FMOC levels (966 (high), 580 (intermediate), and 290 (low) pmol/μL). All WSs of amines and FMOC were prepared and stored in 1.5 mL vials (capacity 1.5 mL, opaque glass, septum capped; Agilent Technologies, USA) for comparative analysis.

The products of amine-FMOC derivatization reaction

A schematic of the derivatization reaction of MA, DMA, and TMA with FMOC is shown in **Fig. 1**. The amine derivatization reaction proceeds via a tetrahedral (quaternary ammonium) intermediate yielding carbamate products (proposed scheme) either by (a) stabilization of intermediate (salt formation) as in the case of TMA, (b) dealkylation (generally very slow for TMA at 25°C) or (c) deprotonation (generally fast under basic condition for MA and DMA) [33]. As shown in **Fig. 1**, the dealkylation of the initially formed TMA-FMOC derivative **(Rxn 1)** can yield an identical FMOC derivative product **(Rxn 2)** as DMA **(Rxn 3)** [24, 30]. However, the dealkylation of the TMA-FMOC acylammonium salt is slow at room temperature and hence the DMA-FMOC carbamate product is insignificant. As a result TMA is detected as an acyl ammonium salt and eluted before the carbamates of MA and DMA.

In the derivatization of three amines altogether, simultaneous acid (hydrochloric acid, HCl) production may be one of the key factors influencing the FMOC-amines derivatization process [9, 34]. Note that HCl is only produced in the derivatization reaction of MA and DMA with FMOC, which subsequently protonates the less basic TMA if (a) MA and DMA (the most basic) are in large molar excess over TMA and (b) more importantly, the TMA/FMOC reaction may be much slower compared to MA or DMA/FMOC reaction. The basicity order in ACN is DMA ($pK_b = 18.7$) > MA ($pK_b = 18.4$) > TMA ($pK_b = 17.6$) [35]. If MA and DMA are in large molar excess over TMA and the unprotonated TMA-FMOC derivatization reaction is slow, TMA will be protonated and hence unreactive toward FMOC. [24] In protonated TMA, the N lone pair is now unavailable in the initial SN_2 attack as shown in **Fig. 1, Rxn 1** and hence derivatization with chloroformates is suppressed as observed for TMA reaction with FMOC in the present work. This is explained and discussed in depth in a review by Szulejko and Kim. [24] If all neutral TMA is removed from solution as protonated TMA, no further TMA-FMOC reaction can occur in the mixture and hence suppressed formation of the FMOC-TMA derivative. This phenomenon was observed and discussed in section 3.2 of Results and discussion.

Injection and analysis of the products from derivatization

After amine derivatization in 1.5 mL vials, 20 µL aliquots were injected onto the HPLC-column by a microsyringe (SGE, Australia) via a 20 µL sample injector loop (Lab Alliance 500). After injection, amine derivatives were separated on a Hichrom 5 C_{18} analytical column. The flow rate was maintained at 1.5 mL/min, while the back pressure (low ~ high) was 0~6000 psi **(Table 1)**. The relative ordering of retention times for all three amine derivatives and FMOC was: acylammonium salt (TMA: 3 min) < carbamate (MA: 3.4 min) < carbamate (DMA: 4.75 min) < free FMOC (7 min) **(Fig. 2C)**.

Construction of calibration curves

For constructing the calibration curves, chromatograms were acquired using a computerized data acquisition and integration system (ds CHROM). In the data acquisition-system, the relative UV-absorption values were obtained as peak area values. These peak area values were then plotted against injected mass to construct the calibration curve (y = mx + C) with correlation coefficient (R^2) values. Quantification of amines in environmental samples was based on the calibration curves constructed including all three amines in mixture.

Optimization of the headspace sampling procedure

For sampling purpose, 3.75 g of rotten fish (thornback ray: *Raja clavata*) was initially placed in an impinger (Schott Duran, Germany) and left for one hour to facilitate thawing and amine emissions under a constant temperature (25°C). Afterwards, the amines released from fish were swept by nitrogen (N_2: 99.999%) at a flow rate of 200 mL/min for 50 min (pump model: Sibata, MP-500, Japan) and collected into a 10 L polyester aluminum (PEA) bag (Top-Trading Company, Korea) **(Fig. 3 (A))**.

For FMOC derivatization, four aliquots (0.50, 1.00, 2.00, and 5.00 L) of collected headspace sample were pulled (at a constant rate of 100 mL/min) through a train of three impingers (prepared freshly for each aliquot) **(Fig. 3 (B))**. Each absorption impinger contained 20 mL of 0.004 M FMOC solution for capturing the gaseous amines through FMOC-derivatization. Triplicate 1.0 mL samples were then taken from each absorption impinger and stored in 1.5 mL vials (opaque glass) (Agilent Technologies, USA) for HPLC analysis.

Results and discussion

Basic properties and reaction kinetics of amine-FMOC derivatization

For HPLC analysis, chromophoric derivatization is a potent option to improve separation and detection of target compounds. FMOC is widely recommended as derivatization reagent for amines as its derivatized products are much more polarizable and have highly chromophoric properties. At initial stage, main goals were set to gain an insight into the basic properties of derivatization reagent (FMOC) along with the purchased chemicals (e.g., acetonitrile). To this end, the blank occurrence pattern of FMOC (without amines) was tested through ten point calibration **(Exp. 1)**.

As presented in **Fig. 2**, TMA-FMOC derivative appeared at around 3 min, while free FMOC eluted later at 7 min. In the analysis of FMOC alone, the retention time of an impurity coincided with that of TMA **(Fig. 2 (A)).** The peak area of the detected TMA impurity in acetonitrile-FMOC was essentially independent of FMOC concentration over a wide range (103 to 30924 pmol in 20 μL of injected standard) **(Table 2 (A))**. Based on this observation, acetonitrile was suspected to be a potential source of TMA impurity. Normally, raw acetonitrile is obtained as a by-product in the industrial production of acrylonitrile with a wide range of impurities (e.g., aliphatic amines), passed through different chemical vendors/treatment processes, and finally bottled for laboratory use.[36-39] The UV spectrum and blank gradient chromatograms of acetonitrile can be evaluated to assess its purity level.[40-42] Another study also reported the absence of impurities if FMOC solution was prepared in distilled water instead of acetonitrile.[13] In our study, to remove the effect of impurity ghost peak, all TMA peak areas at all concentrations (**Exp. 2 - 5** and environmental analysis) were corrected by subtracting the blank peak area value of ~$1.6x10^5$ (0.95 pmol/μL, 0.31 ppm (w/w), or 19 pmol of TMA in each injection of 20 μL FMOC-acetonitrile standard)) **(Table 2)**.

At the next step, the reaction kinetics and temporal variation of amine-FMOC reaction were studied by analyzing the TMA-FMOC derivatization over time **(Exp. 2)**. To this end, two different types of TMA-FMOC derivatization standards were prepared; (A) equimolar TMA and FMOC and (B) 1:2 molar ratio of TMA and FMOC **(Table 2 (B))**. After preparation, 20 μL aliquots of the FMOC derivatization standard were injected at regular intervals on the HPLC system to monitor the attainment of a steady state. For both equimolar (1:1) and 1:2 molar standards, derivatization increased gradually with time and attained a steady state in about 35 and 40 min, respectively **(Fig. 4)**.

It was also interesting to note that the nearly complete derivatization (attainment of applied concentration of TMA (12.5 pmol/μL) by conversion) was observed at an initial (1:2) molar standard ratio of TMA and FMOC. Under the light of these observations, the amount of FMOC **(in Exps 3 and 4)** was chosen to have the initial amine-FMOC molar ratio greater than 1:2.

Derivatization potential of FMOC between amines

To provide an insight into the derivatization potential of FMOC among different amines, we compared results of calibration experiments made by using standards of amines prepared both individually **(Exp. 3)** and as a mixture **(Exp. 4)**. Eight different standards of amines were prepared both individually and as a mixture and injected on the HPLC system (20 μL) for constructing the calibration curves. Peak areas for different amount of amines were obtained to allow comparison of their response factor (RF) values in both approaches **(Table 2 (C) and (D))**.

Fig. 5 depicts the calibration results of three amines for both types of standards: (A) three individual amines and (B) a mixture of three amines. In case of the former, TMA exhibited the highest RF (peak area (au) mol^{-1}) value (7593) among all three amines (MA: 3065 and DMA: 4355). In mixture, lower RF of MA (2896) and TMA (3732) was observed, while RF value of DMA (5454) was higher than previous. Comparison of RF values between these two experiments (individual amines vs. mixture) indicates that the sensitivity of TMA underwent a significant drop (~2 times) under competing conditions, whereas it was not so large for MA (-7.7%). In case of DMA, enhanced detection (25%) was observed. This observation thus suggests the possible suppression in the TMA derivatization, if derivatization proceeds in the presence of other amines as discussed above in the "The products of amine-FMOC derivatization reaction" section. The suppression of TMA in a mixed standard is intimately related to some factors controlling the aminolysis reaction (e.g., amine pK_b, solvent type, and pH). However, the simultaneous production of hydrochloric acid (through the derivatization reaction of MA and DMA with FMOC) may also be one of the key factors influencing the FMOC-amine derivatization process. Controlling pH by using buffer solution may be one possible option to minimize this effect.[16]

Finally, TMA was analyzed at three different concentration levels of derivatization standards (FMOC) to assess the derivatization efficiency vs. FMOC/TMA ratio **(Exp. 5)**. To facilitate this process, five point calibrations were done for TMA at three different concentration levels (low, intermediate, and high) of FMOC **(Table 2 (E))**. Calibration results were than compared on the basis of FMOC concentration levels **(Fig. 6)**. The response of TMA was almost the same at three different FMOC concentrations (low (3520), intermediate (3587), high (3534)). The RF values of free FMOC were in a range of 3673 (I)-4671 (L) **(Fig. 6 (B)).** As excess amount of FMOC was used for derivatization in all the three approaches, it was realistic to obtain almost same response. From this point of view, the response of TMA is independent from FMOC concentration, if excess FMOC is used for derivatization. In the light of this observation, excess amount of FMOC was used to optimize derivatization condition.

Environmental sample analysis

For our analysis of environmental samples, we selected marine thornback ray (*Raja clavata*) which is one of the most popular fish species consumed (in both fresh and dried/rotten form) on the Korean market. Our sample fish (dried) was purchased from a local market (stored at ambient temperature) near Sejong University, Seoul, Korea and kept frozen until sampling. The analysis was made using 10 L of headspace sample collected from rotten fish placed in an impinger (as stated in Materials and Methods section).

In **Fig. 7 (A)**, the MA and TMA derivative concentration in each FMOC absorption solution (in which different aliquots of headspace sample was absorbed) are plotted as a function of absorption volume. The MA and TMA derivative concentration in the absorption medium (20 mL FMOC solution) increased with increasing gas sample volumes. The overall TMA concentration in sweep gas samples is approximately 190 ppm which is higher than MA (~61 ppm) **(Fig. 7 (B))**. However, DMA was not detected in the headspace **(Fig. 2 (D))**.

The emission rate of MA and TMA from rotten fish was calculated as 0.006 and 0.021 mg/g of fish/min, respectively, considering the total sampling volume (10 L), headspace sampling rate (200 mL/min), and total sample mass placed on impinger (3.75 g). The total volatile basic nitrogen (TVBN) content of analyzed rotten fish in 10 L headspace sample was also calculated on an N mass basis per 100 g of fish. The calculated TVBN of this decayed fish was 38.2 mg N/100 g. This result is comparable with another study concerning the HS-SPME-GC-MS analysis of rotten fish (mangrove snappers), while TVBN were measured in a range of 10.9 - 30.1 mg N/100 g.[43] In another study based on capillary electrophoresis with indirect UV detection, TVBN levels in 100 g of Cod fish extract were reported as 114.5 mg N/100 g.[44].

The capture efficiency of headspace sampling and derivatization was also evaluated by estimating breakthrough of impinger sampling. Assuming that the capture efficiency is independent of concentration, the concentration ratio of TMA between the second and first impinger was used to assess the breakthrough. The capture efficiency (at 1st impinger) for TMA was almost 98%; while it was little lower for MA (93%). Relatively low capture efficiency for MA (<90%) than other amines (e.g., DMA and TMA) was also reported in a previous study based on midget impinger sampling of gaseous amines.[45] In another study, capture efficiency was reported in a range of 95-99% for ammonia and aliphatic amines in water at pH 7.[46] In the analysis of different amines (e.g., MA, DMA, TMA, diethylamine, and triethylamine) in ambient air, amine collection efficiency of 0.05 M H_2SO_4 was reported to reach near 100%.[47] Results of those previous studies also indicate moderate to excellent capture efficiency for environmental amines, as seen in this study.

Basic quality assurance of recent studies

To assess the relative performance of amine calibration between different approaches using two types of standards (individual and mixture), quality assurance experiments were done for both standard types **(Table 3)**. These experiments were conducted by injecting 20 µL of amine standards (e.g., 1 pmol/µL for MA and DMA). The instrumental detection limit (DL) values (obtained under optimized conditions) were then calculated according to US-EPA guidelines.[48] DL values for different amines were in a range of 0.05-0.17 ng. HPLC system exhibited relatively enhanced detection properties for individual analysis of amines (except, MA). In case of TMA, DL from individual analysis (0.05 ng) was clearly better than its mixture counterpart (0.16 ng); as aforementioned, it should reflect the suppression of TMA-FMOC derivatization in mixture standards. DL values expressed as mixing ratios were also calculated (in a range of 0.21 (TMA) - 0.94 (DMA) ppb) for a 100 L gaseous sample absorbed in 20 mL FMOC solution in an impinger assuming ~100% recovery. The reproducibility of calibration experiments were also assessed through the triplicate analysis of same standards used for DL study. In both individual and mixture standards, RSE values slightly varied among amines but generally fell below 1% **(Table 3)**.

The results of this study are comparable with many other HPLC-based studies of amines using FMOC as derivatization reagent. In three individual studies of aliphatic amines using FMOC derivatization, DL values were reported as 750 (MA), 300 (DMA), and 250 ng mL^{-1} (TMA).[15, 49, 50] In another study based on SPME and HPLC analysis, DL values were reported as 5 ng mL^{-1} for both MA and DMA but as large as 250 ng mL^{-1} for TMA.[13]

Concluding remarks

In this research, a series of laboratory experiments were designed and conducted to quantify short chained aliphatic amines through their derivatization with FMOC and HPLC-UV detection. Different issues related to the amine-FMOC derivatization (e.g., process, potential, and also reaction kinetics) were studied as an inseparable part of this research. To facilitate comparison, we analyzed both individual and mixture WSs of all three amines. The calibration results for both types of standards generally showed enhanced sensitivity of TMA in individual analysis, while its response was significantly diminished in a mixture. Hence, excess amount of FMOC was applied to facilitate proper derivatization (maintained in a range of 1:500 (at best) to 1:2 (at least)). A time span of 40 min was also proposed for the steady state conversion (by derivatization) of amines to attain suitable UV chromophores. To overcome the effect of TMA-impurities (e.g., in acetonitrile), we also systematically applied blank corrections. By combining those approaches, we have tried to minimize some limitations regarding simultaneous analysis of all three aliphatic amines, as reported from a number of previous studies. The basic quality assurance parameters (e.g., linearity, sensitivity, accuracy, and reproducibility) achieved by the proposed method are found to be adequate for the environmental analysis of trace level amines.

The method here introduced was successfully applied to real samples. In the course of this study, we made a stepwise approach to combine the first step sampling of sweep gas released from fish sample in an impinger and the second step derivatization of TMA from samples with FMOC contained in a separate impinger system. The capture and derivatization efficiency of this impinger system was satisfactory (93% for MA and 98% for TMA) for environmental analysis. However, the results of our environmental analysis indicate a very high amine emission capacity of rotten *R. Clavata* to yield huge TVBN value with significant emission rate. Considering the frequent consumption of *R. Clavata* in both dried and rotten form, some precaution is suggested if consuming this fish in excess.

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Table 1. Basic information of instrumental system and purchased chemicals.

A. Instrumental setup

1. HPLC apparatus		**2. HPLC column**	
(Pump, 20 µL injection loop, and UV detector)		Model	Hichrom 5 C_{18}
Model	Lab Alliance 500	Column dimensions	250 mm (*l*) × 4.6 mm (id)
UV absorbance and detection wavelength	262 nm	Mobile phase	acetonitrile : distilled water = 7:3
		Particle size	5 µm
Total analysis time	15 min	Flow rate	1.5 mL/min
Data acquisition software	ds CHROM	Pressure (high)	6000 psi
Sample injection volume	20 µL	Pressure (low)	0 psi

B. Basic properties of purchased chemicals [a]

Compounds	Short name	Formula	Molecular weight (g mol^{-1})	Density (ng μL^{-1})	CAS Number
Methyl amine	MA	CH_5N	31.1	8.90E+05	74-89-5
Dimethyl amine	DMA	C_2H_7N	45.1	8.90E+05	124-40-3
Trimethyl amine	TMA	C_3H_9N	59.1	9.30E+05	75-50-3
Fluorenylmethyloxy Carbonylchloride	FMOC	$C_{15}H_{11}ClO_2$	259	-	28920-43-6
Acetonitrile	ACN	CH_3CN	41.1	7.86E+05	75-05-8

[a] All three amines and FMOC were purchased from Sigma- Aldrich, Inc., USA; acetonitrile was purchased from J.T. Baker (USA).

Table 2. Comparison of all (stage 1) types of calibration experiments for amines by FMOC derivatization (all quantities of amines and FMOC expressed in pmol contained in 20 µL standard solution for HPLC injection).[a]

(A) Exp. 1: 10 point Calibration of FMOC alone

Order	Mass (pmol)	Peak area		Order	Mass (pmol)	Peak area	
	FMOC	TMA as impurity	FMOC		FMOC	TMA as impurity	FMOC
1	103	137,159	322,129	6	2,062	148,633	12,543,376
2	206	180,236	921,707	7	4,123	175,064	27,220,339
3	309	154,542	1,437,306	8	8,246	186,793	52,069,343
4	618	171,830	3,136,159	9	16,493	137,448	101,427,537
5	1,031	124,808	5,076,393	10	30,924	228,373	211,559,614

(B) Exp. 2: Reaction kinetics study.

A. Results for equimolar (both 500 pmol) TMA-FMOC derivatization standard				B. Results for 1:2 molar (TMA 250 and FMOC 500 pmol), derivatization standard of TMA and FMOC			
Peak area				Peak area			
Order	TMA [b]	FMOC	derivatization time (min)	Order	TMA	FMOC	derivatization time (min)
1	2,197,133	1,429,292	2	1	954,583	1,293,791	2
2	2,610,207	1,146,906	17	2	1,323,539	1,142,874	22
3	3,141,851	531,053	35	3	1,719,774	645,816	40
4	3,114,381	686,850	51	4	1,675,780	752,131	58
5	3,235,457	552,187	67	5	1,891,494	735,611	90

(C) Exp. 3: Calibration of three amines (MA, DMA, and TMA) standards prepared individually

Order	Mass (pmol)	Peak area					
		Amine-FMOC			FMOC (free)[c]		
	MA/DMA/TMA	MA	DMA	TMA	MA	DMA	TMA
1	20	41,804	82,454	34,234	62,171,491	60,102,006	63,052,027
2	40	86,852	186,674	461,871	62,109,200	58,612,504	63,326,984
3	80	202,196	309,570	1,245,751	61,165,880	56,820,448	60,551,249
4	160	607,617	715,518	1,759,816	60,835,860	59,476,234	58,503,902
5	241	943,785	1,172,898	2,425,315	62,575,739	56,110,221	54,822,740
6	481	1,282,365	2,198,014	4,185,175	60,209,260	58,221,072	56,869,840
7	725	2,153,153	2,933,150	5,781,124	62,069,155	56,559,071	63,052,026
8	965	3,052,200	4,273,619	7,532,678	61,638,260	55,272,685	66,233,182

(D) Exp. 4: Calibration of three amines standards prepared as a mixture

Order	Mass (pmol)		Peak area			
			Amine- FMOC			FMOC (free)[d]
	MA/DMA	TMA	MA	DMA	TMA	
1	20	60	19,986	64,725	668,848	62,637,550
2	40	120	78,607	113,147	883,192	63,326,984
3	80	241	104,714	168,431	1,387,796	64,912,831
4	160	481	161,563	446,968	2,446,642	61,113,985
5	241	722	282,853	725,069	2,648,271	60,583,349
6	481	1,443	867,808	1,881,820	5,940,373	58,250,613
7	725	2,160	1,597,217	2,987,576	7,905,676	54,877,907
8	965	2,860	2,980,945	5,509,366	11,453,295	50,787,659

(E) Exp. 5: Calibration of TMA with three different FMOC concentration levels (low, intermediate, and high).

Order	Mass (pmol)	Peak area					
		TMA-FMOC [e]			FMOC (free)		
	TMA	FMOC (H)	FMOC (I)	FMOC (L)	FMOC (H)	FMOC (I)	FMOC (L)
1	100	1,410,663	1,108,887	823,200	124,424,837	71,536,742	35,941,075
2	200	1,921,451	1,249,265	830,443	125,099,284	71,902,274	35,821,220
3	400	2,732,963	2,257,017	1,652,813	118,896,773	70,514,667	35,530,942
4	800	3,617,164	3,480,263	3,142,108	117,533,893	69,336,890	32,792,896
5	1500	6,550,614	6,086,125	5,585,537	117,294,237	64,888,262	27,475,319

[a] All Exps 1 through 5 are made by injecting 20 µL of liquid standards.
[b] The peak areas of TMA were corrected by subtracting the background value (164,489) to minimize the effects of impurities.

Superscript c and d indicate initial amount (in 20 µL injection) of fixed FMOC of 10,060 pmol in preparation of both individual (A) and mixture (B) amine standards in Exps 3 and 4, respectively.

[e] Capital letters H, I, and L in the parenthesis are used to denote the amount of FMOC added to induce derivatization of TMA: high (19,340 pmol), intermediate (11,600 pmol), and low (5,800 pmol), respectively.

Table 3. Detection properties of the LC system employed for the analysis of amines

Properties	(a) Individual standards of amines			(b) Mixture standard of amines		
	MA	DMA	TMA	MA	DMA	TMA
(i) Detection limit: (DL: ng) [a]	0.12	0.08	0.05	0.11	0.17	0.16
(DL: pmol/μL)	0.19	0.09	0.04	0.17	0.19	0.15
(DL: ppb) [b]	0.91	0.43	0.21	0.84	0.94	0.68
(ii) Reproducibility (RSE: %) [b]	0.58	0.96	0.91	0.41	1.29	0.82

[a] DL in ng were calculated for 20 μL injection volume

[b] DL for gaseous standards were based on 100 L gaseous sample absorbed in 20 mL FMOC solution in an impinger assuming ~100 % recovery.

[c] Triplicate analyses by injecting 1 pmol/μL (except TMA in mixture, 2 pmol/μL) standard of all three amines.

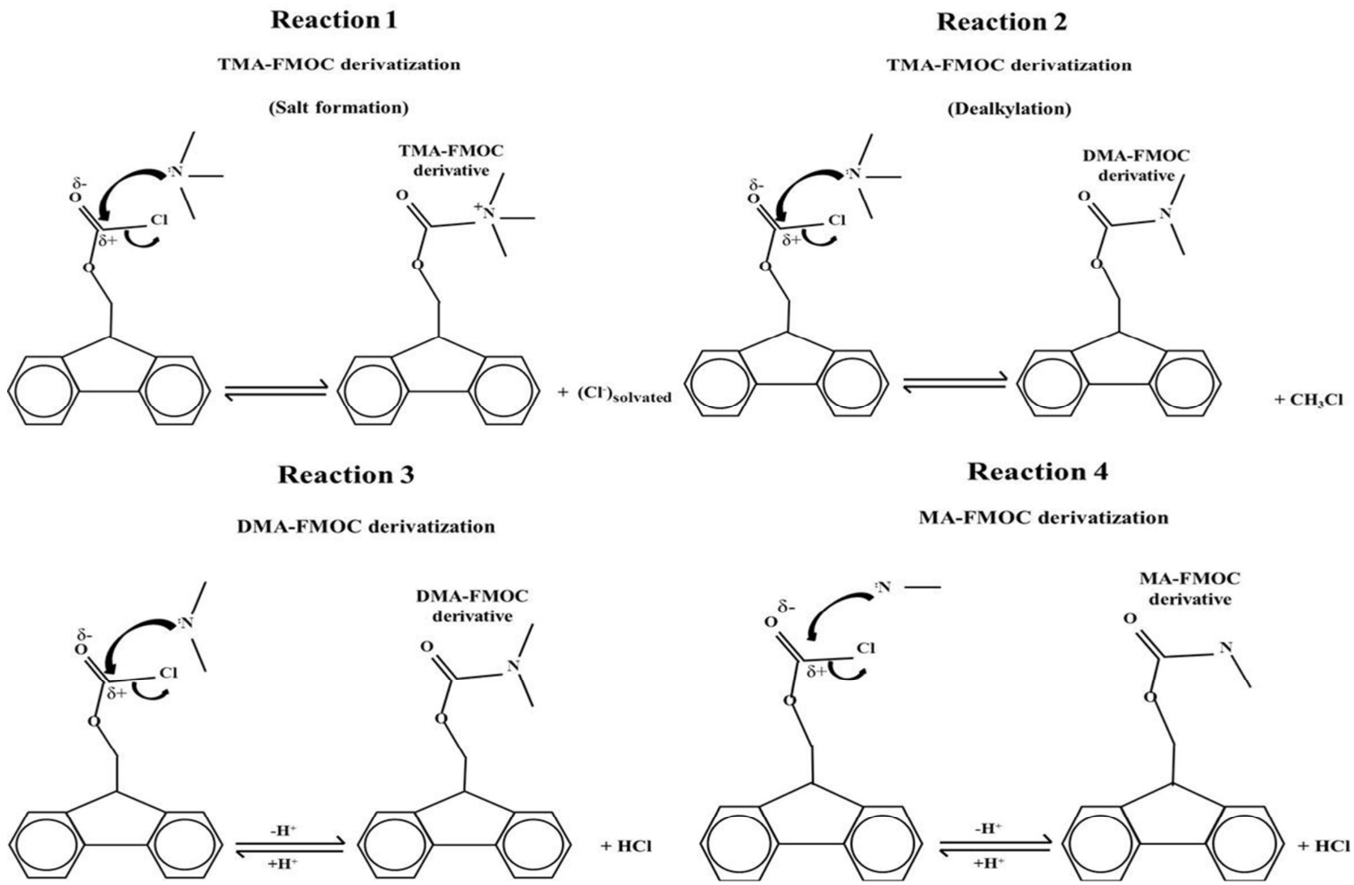

Fig. 1. Proposed reaction scheme of amine-FMOC derivatization: SN_2 reaction mechanism for all three amines.

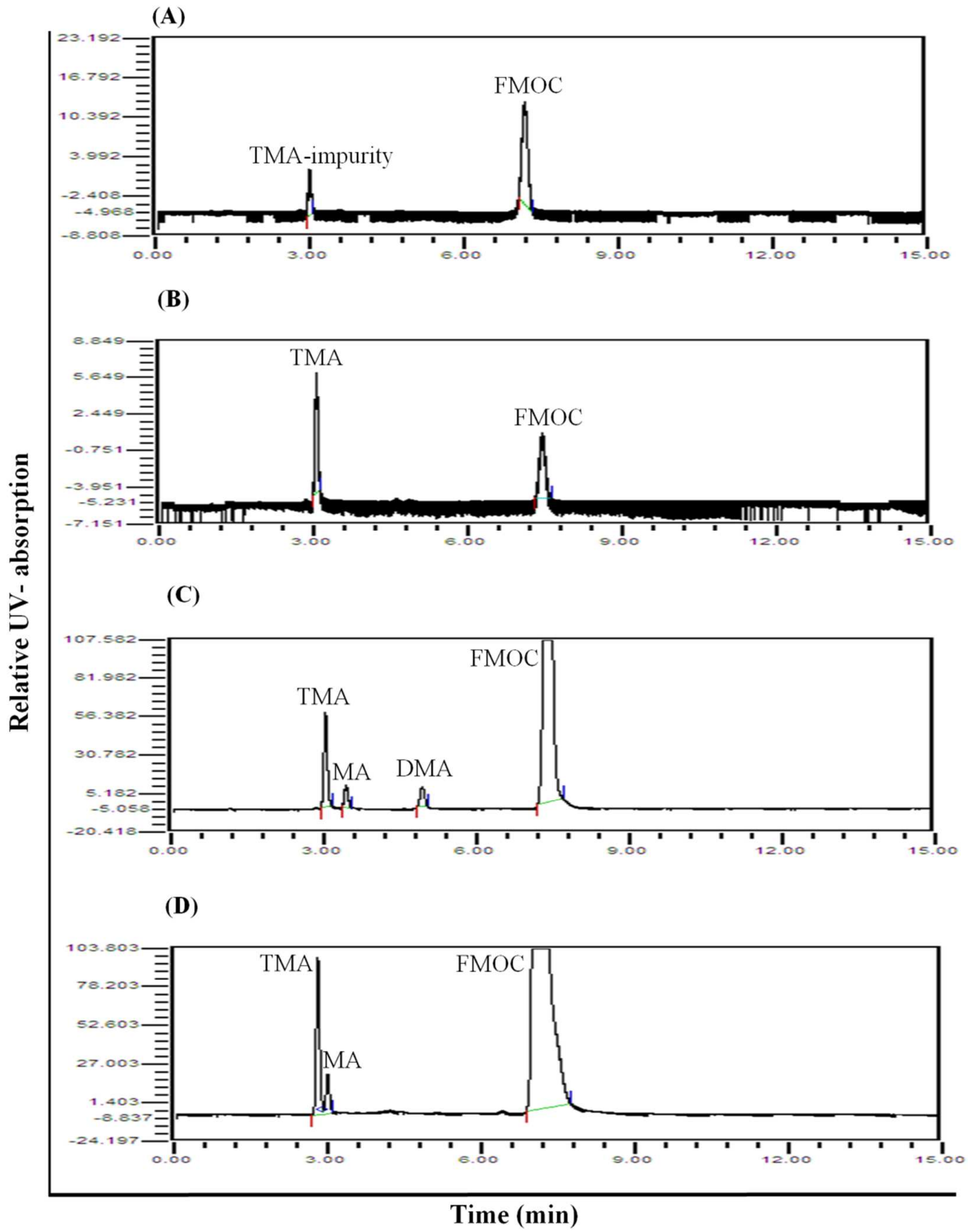

Fig. 2. Representative chromatograms of different experiments:

(A) FMOC solution prepared in acetonitrile (FMOC-31 pmol/μL: **Exp 1**),

(B) TMA-FMOC derivatization standard (TMA-12.5 pmol/μL; FMOC-25 pmol/μL: **Exp 2**)

(C) Mixture standard of all three amines (concentration of MA, DMA, and TMA-24.1, 24.1, and 72.1 pmol/μL, respectively; FMOC-503 pmol/μL: **Exp 4**)

(D) Environmental sample (0.5 L headspace absorption sample) **(Exp stage 2)**.

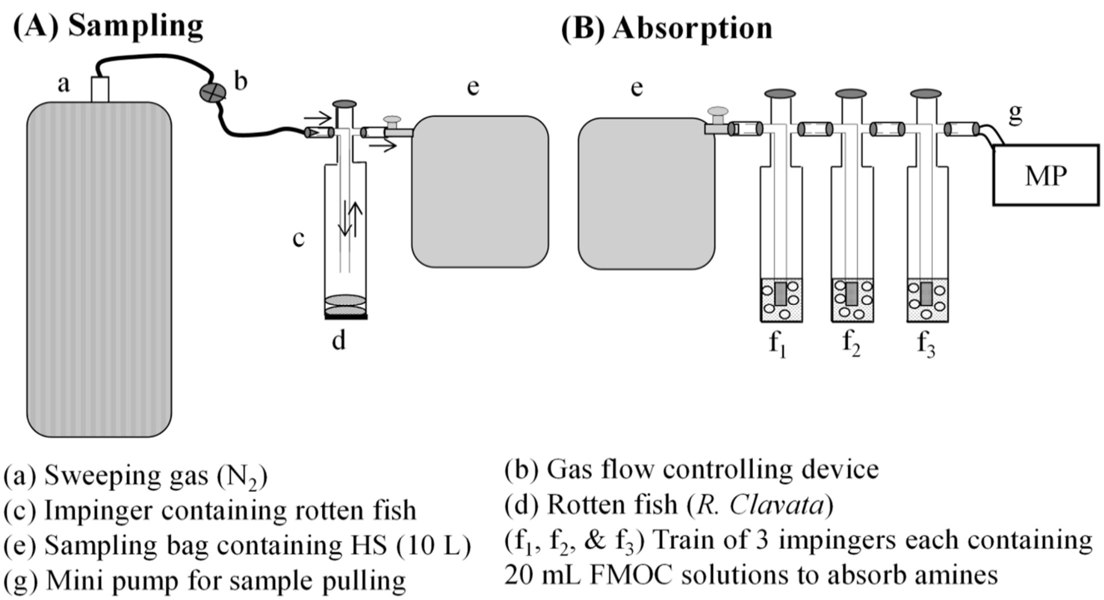

Fig. 3. Schematic representing the arrangement involved in environmental sample analysis: **(A)** Headspace sampling and **(B)** Absorption of amines in FMOC solution though derivatization

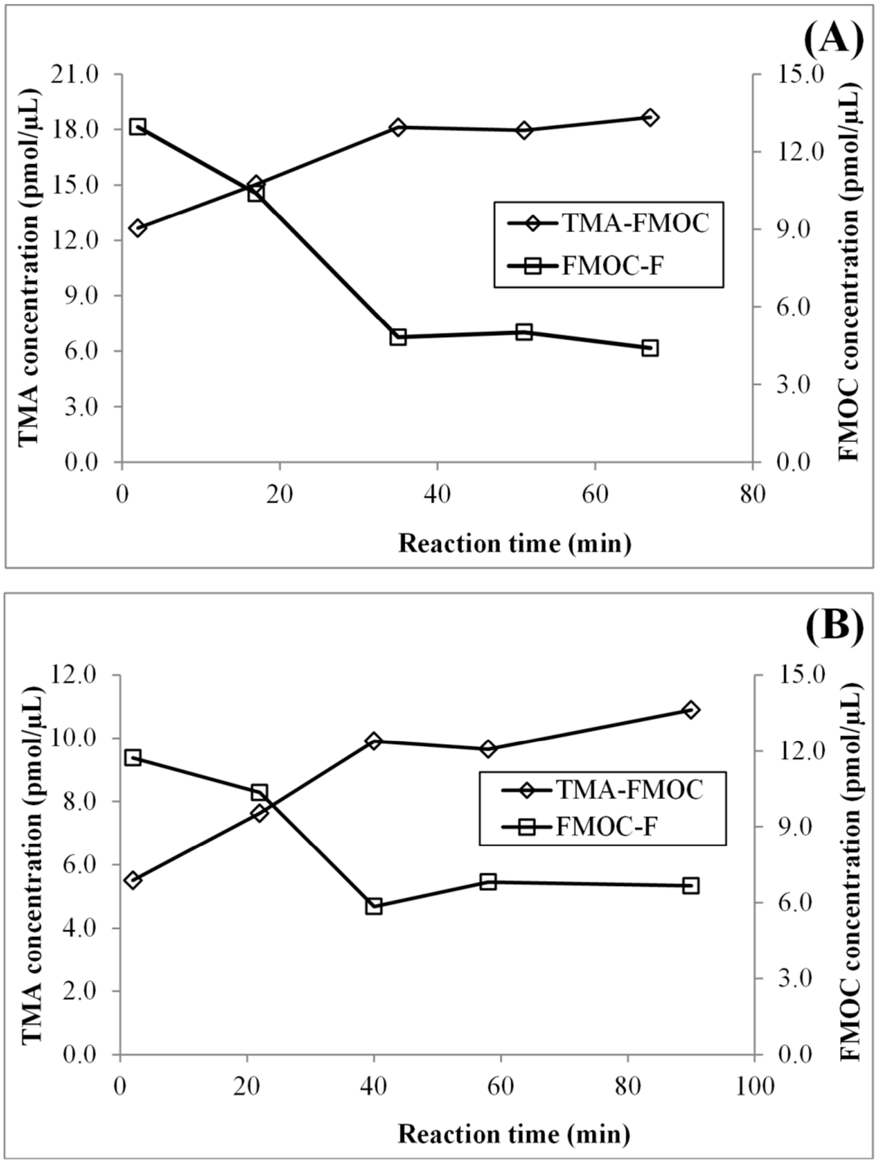

Fig. 4. Time intensity plot of the derivatization reaction between TMA and FMOC. The concentration (pmol/µL) ratio of TMA and FMOC was **(A)** 1:1 (25:25) and **(B)** 1:2 (12.5:25)

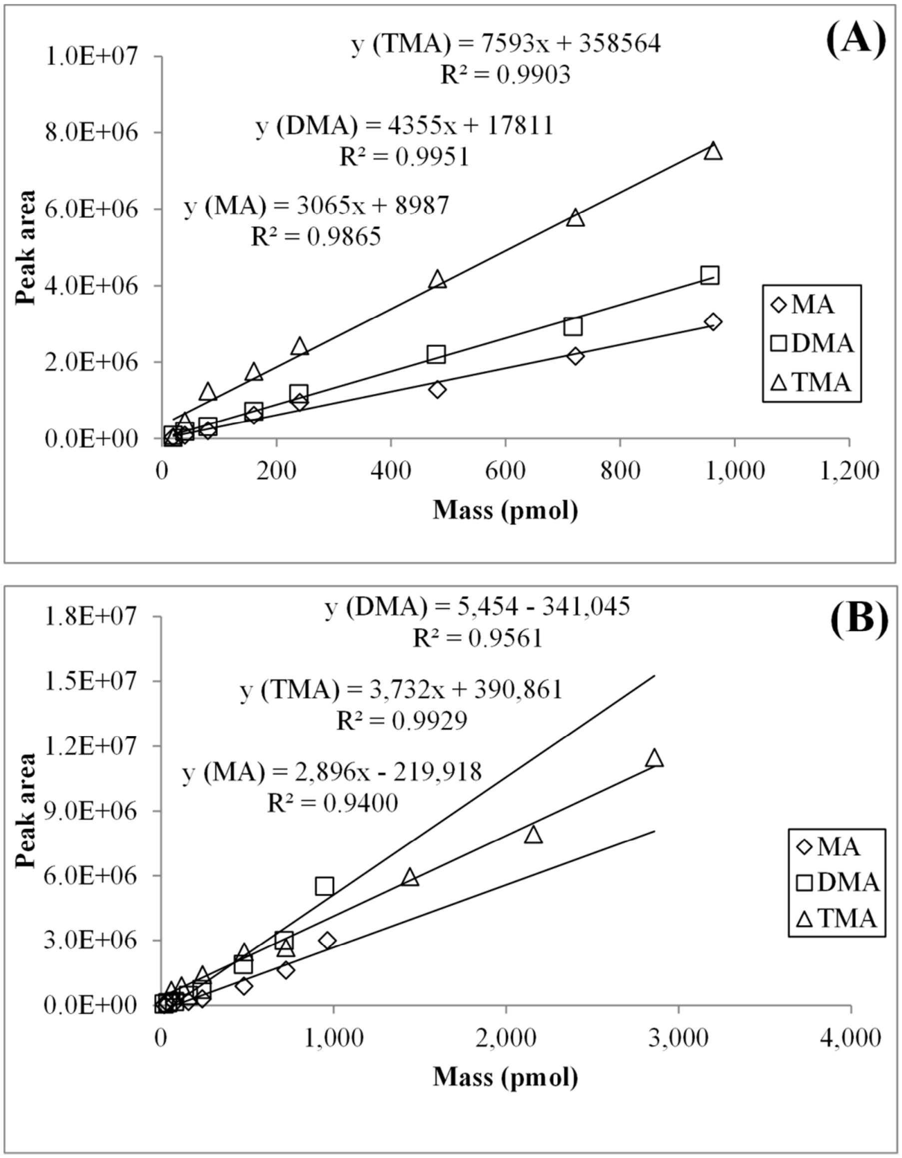

Fig. 5. Calibration results of three amines using standards of **(A)** each of three amines prepared individually (**Exp 3**) and **(B)** Calibration of each amine in presence of another two amines **(Exp 4)**.

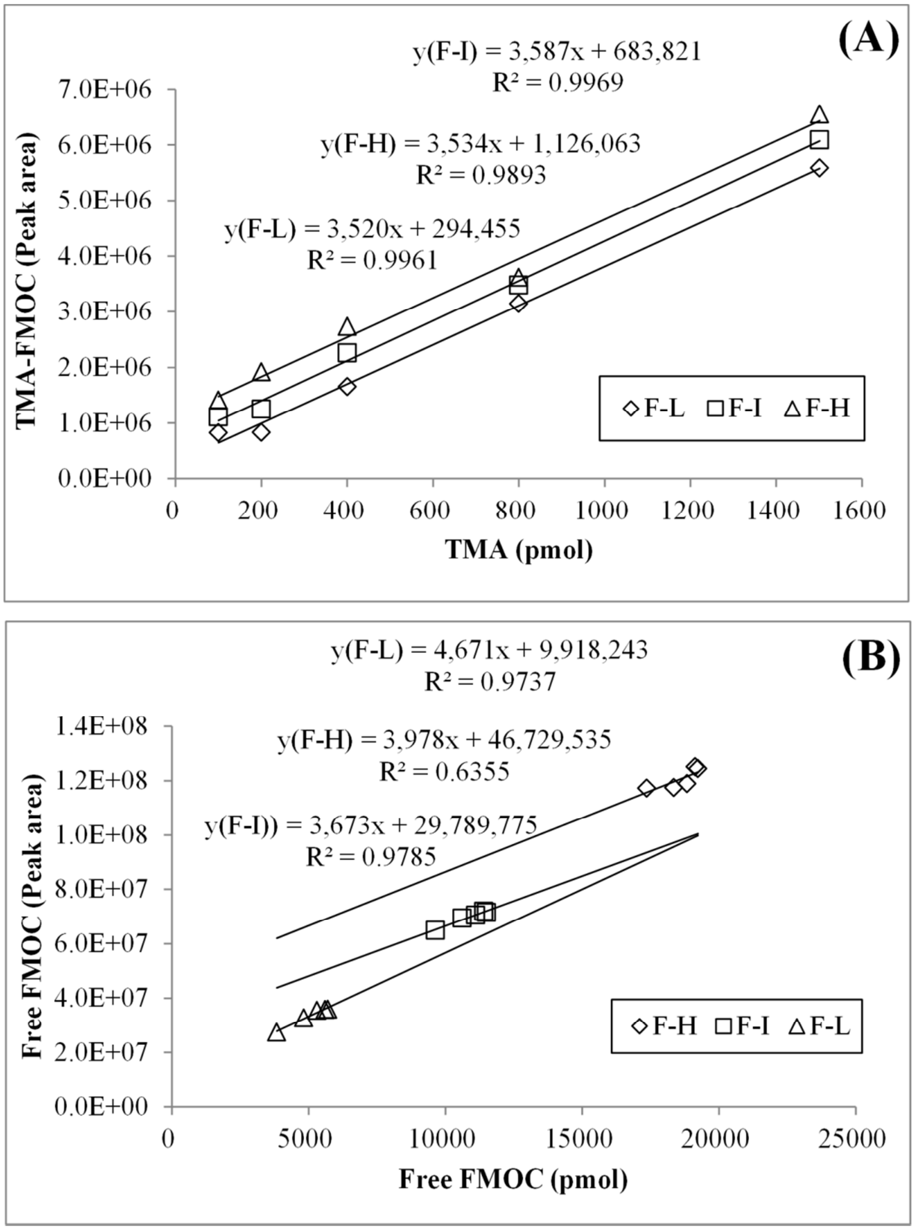

Fig. 6. Comparison of calibration properties of TMA in relation to FMOC concentration levels **(Exp 5)**: **(A)** TMA with three different FMOC levels and **(B)** Free FMOC.

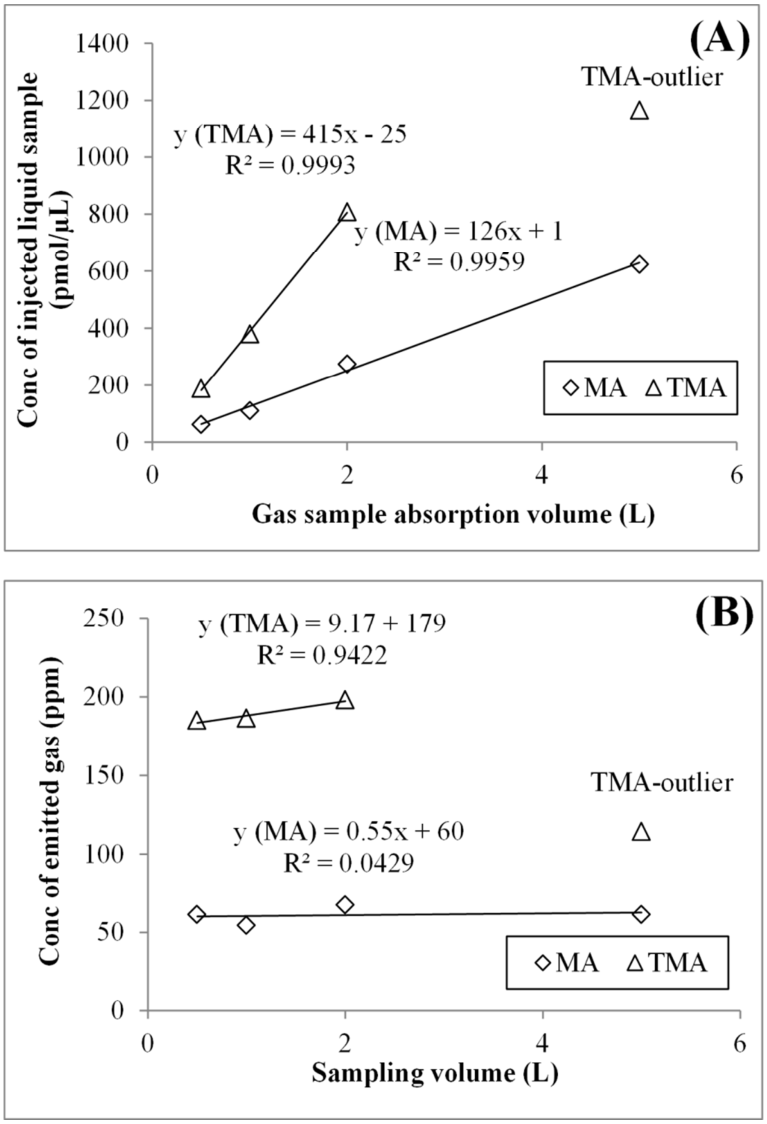

Fig. 7. Dynamic headspace analysis of rotten fish: **(A)** concentrations of captured MA and TMA (pmol/μL, in 20 mL FMOC absorption solution) vs. sampling volume and **(B)** concentration of emitted gas (ppm) vs. sampling volume.

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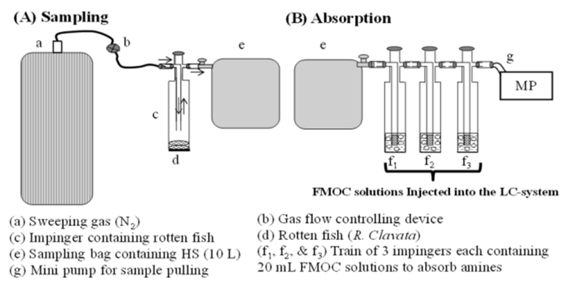
Highlights of the paper in brief

FMOC-based derivatization approach was developed to analyze gaseous amines by HPLC/UV. An impinger-based headspace collection and amine-derivatization system is also described.



Schematic of impinger-system for headspace environmental analysis