Macedonian Journal of Chemistry and Chemical Engineering, Vol. **43**, No. 2, pp. 181–193 (2024)

MJCCA9 – 905

ISSN 1857-5552
e-ISSN 1857-5625

Received: August 12, 2024
Accepted: October 29, 2024

DOI: 10.20450/mjcce.2024.2939
Original scientific paper

ESTABLISHMENT, EVALUATION, AND APPLICATION OF A GAS CHROMATOGRAPHY–MASS SPECTROMETRY METHOD FOR QUANTIFICATION OF VOLATILE ORGANIC COMPOUNDS IN AMBIENT AIR USING ANISOLE AS AN INTERNAL STANDARD[1]

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Gas chromatography coupled with mass spectrometry (GC-MS) is a standard analytical method for identifying specific volatile organic compounds (VOCs) in urban air samples. Quantification ideally relies on calibration curves using standards for each compound. However, due to the variable composition of VOCs and the limited availability of all standards, this strategy is challenging and not always feasible. Quantification using an internal standard is a simple and practical approach, as it involves simultaneous analysis of the internal standard and analytes, ensuring consistency and reliability in the results. In this study, quantification of VOCs from different chemical classes has been evaluated using a single standard mixture and anisole as an internal standard and compared to the external calibration as a standard method for quantification by GC-MS. Both methods showed comparable results, with a relative error within ± 30 % of the theoretical concentration. Linearity was confirmed in the range from 100 to 4000 μg/l, and the relative standard deviation (RSD) of the relative response factors (RRFs) met the acceptance criteria of ≤ 30 % for each target VOC. Toluene, chlorobenzene, C_6-C_2- and C_6-C_3-substituted benzenes yielded similar average RRFs, ranging from 1.280 to 1.601, with an RSD of 7.83 %. The methods were applied for the quantification of VOCs in ambient air using real samples collected on Radiello adsorbents at an urban location. Using anisole as an internal standard proved to be a simple and reliable method for determining VOC concentrations, offering a convenient alternative to external standard calibration.

Keywords: volatile organic compounds; anisole; internal standard; external calibration; GC-MS

ВОСПОСТАВУВАЊЕ, ПРОВЕРКА И ПРИМЕНА НА МЕТОД ЗА КВАНТИФИКАЦИЈА НА ИСПАРЛИВИ ОРГАНСКИ СОЕДИНЕНИЈА ВО АМБИЕНТАЛЕН ВОЗДУХ СО ПРИМЕНА НА ГАСНА ХРОМАТОГРАФИЈА СО МАСЕНА СПЕКТРОМЕТРИЈА И АНИЗОЛ КАКО ВНАТРЕШЕН СТАНДАРД

Гасната хроматографија спрегната со масена спектрометрија (анг. GC-MS) е стандарден аналитички метод за идентификација на специфични испарливи органски соединенија (анг. volatile organic compounds, VOCs) во примероци од урбан воздух. Нивната квантификација во идеален случај се заснова на калибрациони прави добиени со стандард за секое соединение. Сепак, поради променливиот состав на VOCs и ограничената достапност на сите стандарди, оваа стратегија не е секогаш остварлива. Квантификацијата со примена на внатрешен стандард е едноставен и практичен пристап, бидејќи вклучува истовремена анализа на внатрешниот стандард и аналитите, обезбедувајќи конзистентност и веродостојност на резултатите. Во ова истражување е извршена евалуација на квантификацијата на испарливи органски соединенија од различни хемиски класи со користење смеса од стандарди и анизол како внатрешен стандард и споредена е со надворешната

[1] Dedicated on the occasion of the Golden Jubilee of the *Macedonian Journal of Chemistry and Chemical Engineering*

калибрација како стандарден метод за квантификација со GC-MS. Двата метода покажуваат споредливи резултати со релативна грешка од ±30 % во однос на теоретската концентрација. Потврдена е линеарноста во опсег од 100 до 4000 μg/l, а релативната стандардна девијација (RSD) на релативните фактори на одговор на детекторот (анг. relative response factors, RRF) е во согласност со критериумите за прифаќање од ≤30 % за сите целни испарливи соединенија. Толуен, хлоробензен, C_6-C_2- и C_6-C_3-супституираните бензени даваат слични просечни вредности за RRF, во опсег од 1,280 до 1,601, со RSD од 7,83 %. Методите се применети за квантификација на испарливи органски соединенија во амбиентален воздух со користење на реални примероци собрани на Radiello-ви комерцијални атсорбенти на урбана локација. Употребата на анизол како внатрешен стандард се покажа како едноставен и веродостоен метод за одредување на концентрациите на испарливите органски соединенија, нудејќи практична алтернатива наместо калибрација со надворешен стандард.

Клучни зборови: испарливи органски соединенија; анизол; внатрешен стандард; надворешна калибрација; GC-MS

1. INTRODUCTION

Volatile organic compounds (VOCs) are common air pollutants in urban environments, significantly impacting air quality worldwide and contributing to the formation of tropospheric ozone, smog, and secondary organic aerosols.[1–3] This can lead to adverse effects on climate, the biosphere, and human health.[4] VOCs represent a diverse group of organic chemicals, including aliphatic and aromatic hydrocarbons and their halogenated derivatives, alcohols, aldehydes, ketones, esters, and terpenes. The ubiquitous presence of VOCs in the environment, originating from both anthropogenic and natural sources, has been a subject of increasing research interest over recent decades.[5,6]

Although numerous substances are classified as volatile organic compounds (VOCs), the most prevalent in urban environments are benzene and its derivatives, including toluene, ethylbenzene, and xylenes (*o*-, *m*-, and *p*-), collectively referred to as BTEX. These compounds constitute more than 60 % of the VOCs detected in urban environments[4] making them essential reference points for assessing environmental levels and exposure to VOCs.[5]

Qualitative and quantitative analysis of VOCs in ambient air is challenging due to their diversity and low concentrations (pg/l to μg/l). Typically, this analysis is conducted using gas chromatography coupled with flame ionization detection (GC-FID)[3,5,6] or mass spectrometry (GC-MS).[3,4,6,7] The US Environmental Protection Agency (EPA) introduced standard methods for toxic organics (TO) in the 1990s, establishing procedures for determining VOCs in ambient air, including methods TO-12, TO-14, TO-15, TO-1, TO-2,[8] and TO-17[9]. These methods generally involve quantification through either calibration curves of all components or by calculating the average relative response factor of each component, based on values averaged across several concentration levels.

However, both of these quantification methods require the preparation of a series of calibration standards at different concentration levels, which is not always preferable since it requires more time, effort, and resources that are not always cheap and readily available. An alternative approach is to use a single standard solution with a known concentration of components, which is a more convenient, time-saving, and resource-efficient method, simplifying the analytical process while reducing costs and minimizing waste. The updated standard methods[10] recommend that analysts use professional judgment when choosing the most suitable calibration model.

1.1. *Quantification approaches*

Choosing the optimal quantification method is a crucial step, as the reliability and consistency of the analytical results depend on it, especially when analyzing trace amounts of compounds in ambient air. The peak height or area in a chromatogram is proportional to the compound's concentration in the solution injected into the gas chromatograph.[11] Several approaches for quantification are possible,[12–14] with methods based on an internal standard and external calibration being the most commonly used in gas chromatography.

External calibration involves running a series of standard solutions with known analyte concentrations and recording the response (e.g., peak area or height). Calibration curves are then constructed by plotting the response against the concentration for each analyte, covering the expected range of analyte concentrations in unknown samples. The analyte concentration in unknown samples can then be determined by comparing their

response to the calibration curve.[11] While straightforward, this method assumes a consistent system response, which may not hold over time, especially in MS, where detector stability can fluctuate between runs, potentially affecting quantification accuracy.[15] A practical way to compensate for system variations is by using internal standard calibration. This involves adding a known amount of a stable, chemically similar internal standard (one that does not interfere with analyte detection) to both standard solutions and unknown samples.[12,14,16] The detector's response to the analyte is then compared to that of the internal standard, allowing calculation of analyte content without needing a calibration curve, as long as both are within the linear range. The main benefit is that it compensates for sample preparation, matrix effects, and instrument variations.

Various studies have employed either internal[15,17–20] or external[21–27] calibration methods, or both,[1,7,28] for analyzing volatile organic compounds (VOCs). However, there is a lack of comparative analysis regarding the overall performance of these two methods in gas chromatography, with very few publications in this area,[29,30] especially for the analysis of VOCs.[31,32] Given the advantages and limitations of both methods, the most effective approach to determine whether to apply external calibration or an internal standard is to evaluate both approaches. Ideally, each compound should have its own labeled or deuterated internal standard. However, due to the high cost of internal standards, a single internal standard can be used for compounds with similar physicochemical or retention properties.[33] Deuterated toluene[15], deuterated chlorobenzene, or fluorinated hydrocarbons (fluorobenzene,[18] 1,4-difluorobenzene,[18] chloropentafluorobenzene, 1-bromo-4-fluorobenzene, and 4-bromofluorobenzene) are commonly used as internal standards for VOCs analysis, as they meet the necessary criteria.[1,10,15,18,19] Anisole has also proven particularly suitable as an internal standard in a previous study,[34] as it is not typically detected in urban environmental samples, has a well-separated peak from other target compounds in the chromatogram, and has a distinctive mass spectrum.

Considering previously published data on the quantification of VOCs in ambient air, the main focus of this work was to evaluate and compare two GC-MS methods for the quantification of VOCs in ambient air: one based on external calibration with a set of calibration standards, and the alternative based on using a single calibration standard mixture with anisole as an internal standard. The study was performed using a model mixture of fourteen different VOCs from different chemical classes, and the results aim to aid researchers and practitioners in selecting the most appropriate method for quantifying these compounds during ambient air monitoring.

2. EXPERIMENTAL SECTION

2.1. *Materials and methods*

2.1.1. *Solvents and standards*

All solvents and standards were of analytical grade. Dichloromethane (≥ 99.8 %, stabilized with amylene, Fisher Chemical, USA) was used as a solvent for preparation of the standard solutions and extraction of the compounds from real samples via solvent desorption.

The compounds listed in Table 1 were selected as target analytes for this study. Two standard solutions containing VOCs from different classes were prepared, mixed, and then used for the preparation of a series of calibration standards. The first was a BTEX standard solution with a concentration of 8000 μg/l, prepared by diluting a BTEX stock solution in hexane (containing benzene, toluene, ethylbenzene, and *o*-, *m*-, and *p*-xylene, each at a concentration of 200 mg/l, CPAchem Ltd, Bulgaria) with dichloromethane. A second standard solution in dichloromethane was prepared through a two-step dilution that contained the following standard compounds, with concentrations ranging from 8410 to 16200 μg/l: trichloroethylene (≥ 99.5 %); tetrachloroethylene (≥ 99.0 %); butyl acetate (≥ 99.7 %); chlorobenzene (≥ 99.5 %); α-pinene (≥ 97.0 %); β-pinene (≥ 99.0 %); 1,2,4-trimethylbenzene (≥ 98.0 %); *p*-cymene (≥ 99.5%); and *R*-(+)-limonene (≥ 98.0 %), all from Sigma-Aldrich, USA. The two solutions were mixed, and a series of calibration standards was prepared at six concentration levels (ranging from 0.10 to 8.00 mg/l) by dilution with dichloromethane. A list of all standards is given in the supplementary Table S1.

Anisole was used as an internal standard. An anisole solution at a concentration 99500 μg/l was prepared through a two-step dilution with dichloromethane. An accurately measured volume of this solution was added to each of the six standard solutions, ensuring a consistent anisole concentration of 1990 μg/l across all standards.

2.1.2. *VOC sampling and sample preparation*

VOCs in ambient air were collected on Radiello diffusive samplers (Fondazione Salvatore

Maugeri, Padova, Italy) filled with activated charcoal, suitable for solvent extraction. To compare the results obtained with both quantification methods, air samples were taken from one location near the Institute of Chemistry in Skopje (42°00'05.0"N, 21°27'12.1"E) in September and November 2022. The diffusive samplers were placed at the location and exposed to ambient air for one month. Sample preparation is described elsewhere.[34] Briefly, anisole was added as an internal standard to the adsorbent, and the retained compounds were desorbed from the adsorbents with dichloromethane. The final extracts were analyzed by gas chromatography coupled to mass spectrometry.

2.1.3. *Gas chromatography analysis*

VOC analysis was performed using an Agilent 8890N gas chromatograph coupled to a 5977B mass spectrometer as the detector. An HP5-MS capillary column (30 m × 0.25 mm × 0.25 μm) with a (5 %-phenyl)-methylpolysiloxane stationary phase was used. The mobile phase was helium, with a purity of > 99.999 %, maintained at a constant flow rate of 1 ml/min. The injection volume was 2 μl in split mode (1:2) with the injector temperature set to 240 °C. Programmed-temperature elution was used, with the column temperature adjusted as follows:

Step (°C/min)	Temperature (°C)	Hold time (min)
	35	5
5	90	3
10	280	2

Electron ionization was used, with the ion source voltage set at 70 eV. The temperatures of the ion source and the detector were maintained at 230 °C and 150 °C, respectively. The mass spectrometer collected data in scan mode, with an m/z range from 35 to 500. Compounds were identified by comparing their retention times with those of commercially available standards, as well as by interpreting their mass spectra and comparing them with the NIST mass spectral libraries (NIST MS Search 2.3).

3. RESULTS AND DISCUSSION

3.1. *External calibration method*

To evaluate this method, parameters such as linearity, precision, limit of detection (LOD), and limit of quantification (LOQ) were calculated. Linearity is defined as the ability of an analytical method to produce results that are directly proportional to the concentration or amount of the analyte within a defined range.[35] To determine linearity, standard solutions were prepared that covered a concentration range expected for the analytes in real samples. A previous study indicated that the estimated concentrations of BTEX and other VOCs[34] in real air samples were around and below 3200 μg/l. Therefore, to test the linearity of the method, six standard solutions of VOCs in dichloromethane were prepared, with concentrations ranging from 100 to 8000 μg/l. Each solution contained a constant concentration of anisole as an internal standard (1990 μg/l). These solutions were then injected and analyzed. A chromatogram of the VOC standard mixture is shown in Figure 1, and the respective list with compound names and retention times is provided in Table 1. Under the experimental conditions, *m*-xylene and *p*-xylene co-eluted, and their combined results were reported, which was expected due to the use of the HP- 5MS column for separation of the analytes.[36]

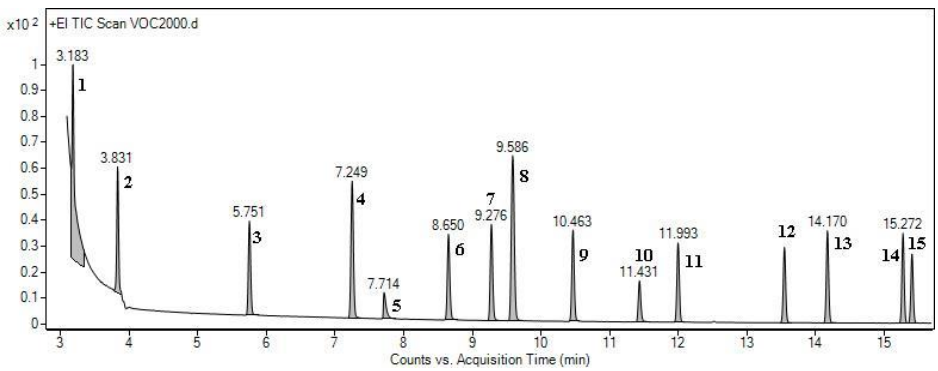


Fig. 1. Chromatogram of a VOCs standard mixture with γ = 2000 μg/l (Compound names and retention times are given in Table 1.)

T a b l e 1

Target VOCs and their retention times (chromatogram in Figure 1)

Peak	Retention time (min)	Name
1	3.183	Benzene
2	3.836	Trichloroethylene
3	5.756	Toluene
4	7.254	Tetrachloroethylene
5	7.746	Butyl acetate
6	8.661	Chlorobenzene
7	9.281	Ethylbenzene
8	9.597	*p*+*m*-Xylene
9	10.468	*o*-Xylene
10	11.436	Anisole (IS)
11	11.998	α-Pinene
12	13.549	β-Pinene
13	14.175	1,2,4-Trimethylbenzene
14	15.272	*p*-Cymene
15	15.405	Limonene

3.1.1. *Linearity*

Quantitative analysis can be carried out based on either peak height or peak area.[11] To determine which method is better, two sets of calibration curves were constructed by plotting: 1) peak area and 2) peak height of each compound in the chromatogram against its concentration. The calibration curve equations and the concentration ranges are given in Table 2. The results obtained from linear regression analysis using peak areas demonstrated comparable or slightly better linearity of the calibration curves across the studied range, with $R^2 > 0.999$ for all analytes except for benzene. The poorer performance of benzene is attributed to the overlapping of the benzene peak in the chromatogram with the intense solvent peak of dichloromethane (Fig. 1), which hampers accurate integration and quantification, particularly at low concentrations. Consequently, better linearity for benzene was obtained by plotting the peak height against concentration.

T a b l e 2

Calibration curve equations obtained by plotting peak area and peak height against concentration of the compound in the standard solution

			Peak area		Peak height	
No.	Component	Concentration range (μg/l)	Calibration curve	R^2	Calibration curve	R^2
1	Benzene	142.5 – 4560	$y = 421.3x + 611773$	0.9765	$y = 141.93x + 98300$	0.9980
2	Toluene	142.5 – 4560	$y = 205.9x – 14322.42$	0.9993	$y = 93.227x – 9716$	0.9992
3	Ethylbenzene	142.5 – 4560	$y = 210.94x – 15082.16$	0.9993	$y = 99.81x – 17343$	0.9983
4	*p*+*m*-Xylene	142.5 – 4560	$y = 420.99x – 21861.25$	0.9998	$y = 159.97x – 17242$	0.9994
5	*o*-Xylene	142.5 – 4560	$y = 191.22x – 13810.94$	0.9990	$y = 97.23x – 19262$	0.9977
6	Trichloroethylene	228.1 – 7300	$y = 143.48x – 8776.36$	0.9997	$y = 123560x – 166085$	0.8866
7	Tetrachloroethylene	253.1 – 8100	$y = 178.81x + 2114.21$	0.9999	$y = 68.821x + 4024.6$	0.9995
8	Butyl acetate	137.8 – 4410	$y = 70.65x – 7401.21$	0.9996	$y = 29.889x – 6111.1$	0.9962
9	Chlorobenzene	173.4 – 5550	$y = 188.25x – 12089.37$	0.9990	$y = 69.159x – 7163.8$	0.9990
10	α-Pinene	134.1 – 4290	$y = 173.25x – 13800.74$	0.9990	$y = 89.382x – 16211$	0.9979
11	β-Pinene	136.3 – 4360	$y = 142.40x – 2913.79$	0.9991	$y = 88.351x – 21672$	0.9959
12	1,2,4-Trimethylbenzene	136.9 – 4380	$y = 179.59x – 14310.03$	0.9990	$y = 94.725x – 15242$	0.9952
13	*p*-Cymene	133.9 – 4285	$y = 163.76x – 5456.59$	0.9990	$y = 101.75x – 22250$	0.9974
14	Limonene	131.4 – 4205	$y = 122.97x – 8741.05$	0.9991	$y = 76.228x – 15159$	0.9990

3.1.2. *Sensitivity and precision*

The sensitivity of the method was determined by using standard solutions with low concentrations of the analytes (ranging from 8 to 50 μg/l), with calibration curves plotted for this low concentration range. The limit of detection (LOD) was calculated using the formula:[35]

$$\mathrm{LOD} = \frac{3.3\,\sigma}{S}, \qquad (1)$$

where σ is standard deviation of the intercept of the calibration curve, and S is the slope of the calibration curve. The limit of quantification LOQ was calculated by using the following formula:[35]

$$\mathrm{LOQ} = \frac{10\,\sigma}{S}\ , \qquad (2)$$

The limits of detection and quantification for the compounds are summarized in Table 3. A repeatability (intraday precision) of 4 – 13 % (expressed as RSD, Table 3) was obtained for the tested compounds through repeated injections of a standard mixture with low concentration of VOCs (8 μg/l). Interday precision (reproducibility) was assessed by performing four repeated injections of a standard mixture across four different days (RSD, Table 3).

T a b l e 3

Limits of detection (LOD), limits of quantification (LOQ), repeatability (n = 7), and reproducibility (n = 4)

Component	LOD (μg/l)	LOQ (μg/l)	Repeatability RSD (%)	Reproducibility RSD (%)
Benzene	6.97	23.00	10.74	8.25
Toluene	3.01	9.12	6.58	5.13
Ethylbenzene	2.85	8.64	11.55	6.99
p+*m*-Xylene	2.77	8.41	10.12	4.64
o-Xylene	2.26	6.86	12.49	10.40
Trichloroethylene	7.49	22.71	6.11	5.56
Tetrachloroethylene	0.28	0.86	4.93	2.09
Butyl acetate	5.46	16.55	10.62	5.90
Chlorobenzene	6.61	20.04	8.39	9.73
α-Pinene	7.83	23.73	9.34	5.08
β-Pinene	8.37	25.37	7.73	6.51
1,2,4-Trimethylbenzene	11.70	35.47	8.93	7.96
p-Cymene	0.21	0.62	7.50	7.73
Limonene	8.51	25.79	8.50	10.62

3.2. *Internal standard method*

This approach is based on the principle that within a specific concentration range, the ratio between chromatographic peak areas and concentrations remains constant. This constant ratio is known as the relative response factor (RRF) for a given component:[10,12]

$$RRF(\mathrm{C}) = \frac{\frac{A(\mathrm{C})}{\gamma(\mathrm{C})}}{\frac{A(\mathrm{IS})}{\gamma(\mathrm{IS})}} = \frac{A(\mathrm{C})}{A(\mathrm{IS})} \cdot \frac{\gamma(\mathrm{IS})}{\gamma(\mathrm{C})}\,, \qquad (3)$$

where A(C) and A(IS) are the peak areas of the target compound C and internal standard IS, respectively, and γ(C) and γ(IS) are their respective mass concentrations. The concentration of each component in the unknown mixture can be calculated by rearranging the above equation:

$$\gamma(\mathrm{C}) = \frac{\gamma(\mathrm{IS})}{RRF(C)} \cdot \frac{A(\mathrm{C})}{A(\mathrm{IS})}\,. \qquad (4)$$

3.2.1. *Linearity*

It is essential to ascertain the value of the response factor in order to obtain reliable analytical results. Furthermore, determining the concentration range in which the peak areas of the analyte and internal standard exhibit a linear relationship is crucial, as only under these conditions can the constancy of the response factor be guaranteed.[10,37]

To verify linearity and determine the relative response factors for the components, standard mixtures containing known amounts of each of the components and the internal standard were analyzed. Linearity was evaluated by plotting the area ratio A(C)/A(IS) against the concentration ratio γ(C)/γ(IS) for each concentration level. The range of the mass concentrations of the analytes is given in Table 2, with the mass concentration of the internal standard (anisole), γ(IS), being consistent across all solutions (1990 μg/l). The regression data are given in Table 4, demonstrating that the coefficient of determination (R^2) for the calibration curves of all target VOCs meets the requirements, with values ≥ 0.995.[10]

T a b l e 4

Calibration curve equations obtained by plotting area ratio A(C)/A(IS) against concentration ratio γ(C)/γ(IS), and average RRF and RSD for each RRF for each analyte

No.	Component	Calibration curve	R^2	Average RRF	RSD (%)
1	Benzene	$y = 3.5703x$	0.9526	4.7464	32.3
2	Toluene	$y = 1.5771x$	0.9997	1.5293	9.72
3	Ethylbenzene	$y = 1.615x$	0.9993	1.5438	15.8
4	*p*+*m*-Xylene	$y = 3.2446x$	0.9995	1.6011	19.4
5	*o*-Xylene	$y = 1.4631x$	0.9998	1.4623	17.4
6	Trichloroethylene	$y = 1.1111x$	0.9992	1.8108	6.30
7	Tetrachloroethylene	$y = 1.406x$	0.9980	2.5480	6.69
8	Butyl acetate	$y = 0.5345x$	0.9976	0.4646	21.3
9	Chlorobenzene	$y = 1.4499x$	0.9998	1.5396	8.32
10	α-Pinene	$y = 1.32x$	0.9997	1.4623	17.4
11	β-Pinene	$y = 1.1087x$	0.9991	1.1456	22.4
12	1,2,4-Trimethylbenzene	$y = 1.3692x$	0.9998	1.3564	23.8
13	*p*-Cymene	$y = 1.2688x$	0.9995	1.2803	20.5
14	Limonene	$y = 0.9395x$	0.9995	0.9242	29.2

3.2.2. *Sensitivity and precision*

The limits of detection and quantification obtained using the internal standard calibration method are given in Table 5. These values are comparable to those achieved with the external standard calibration method, with the exception of benzene. For benzene, lower limits of detection and quantification were achieved using the external calibration method.

Concentrations of compounds in air are typically measured as mass per volume of air, and the limits of detection and quantification are therefore expressed in the same manner; however, these values are dependent upon the volume of the air sample. In our study, the concentration of toluene after 7 days of exposure of the Radiello sampler, considering its sampling rate at 25 °C,[38] resulted in a limit of detection of 0.03 μg/m^3, which aligns with previously reported values.[39] The EPA, using IRIS (Integrated Risk Information System) data, has established reference concentrations (RfCs) for various air pollutants:[4] 0.03 mg/m³ for benzene (associated with hematological effects); 5.0 mg/m³ for toluene (neurological and liver effects); 1.0 mg/m³ for ethylbenzene (respiratory, liver, and kidney effects); and 0.1 mg/m³ for xylenes (respiratory, neurological effects, and low birth weight). Chronic exposure to benzene at 0.03 mg/m³ increases the leukemia risk to 1 in 10,000.

The limits obtained through the internal standard method for the target analytes are lower than the RfCs. Additionally, the estimated limits of detection are below the maximum acceptable toxic concentration (MATC) values for ethylbenzene (770 μg/m³), toluene (400 μg/m³), and total xylenes (870 μg/m³). Since the estimated detection and quantification limits for these compounds fall below the EPA's RfCs and the MATC values, this indicates that the method can detect pollutant levels below regulatory thresholds. However, external calibration should be used for the quantification of benzene to achieve a limit of detection lower than the MATC.

A precision of 5 – 15 % (expressed as RSD, Table 5) was obtained for the tested compounds using repeated injections of a standard mixture with a low concentration of VOCs (8 μg/l).

T a b l e 5

Limits of detection (LOD), limits of quantification (LOQ), and precision (n = 7) of the internal standard calibration method

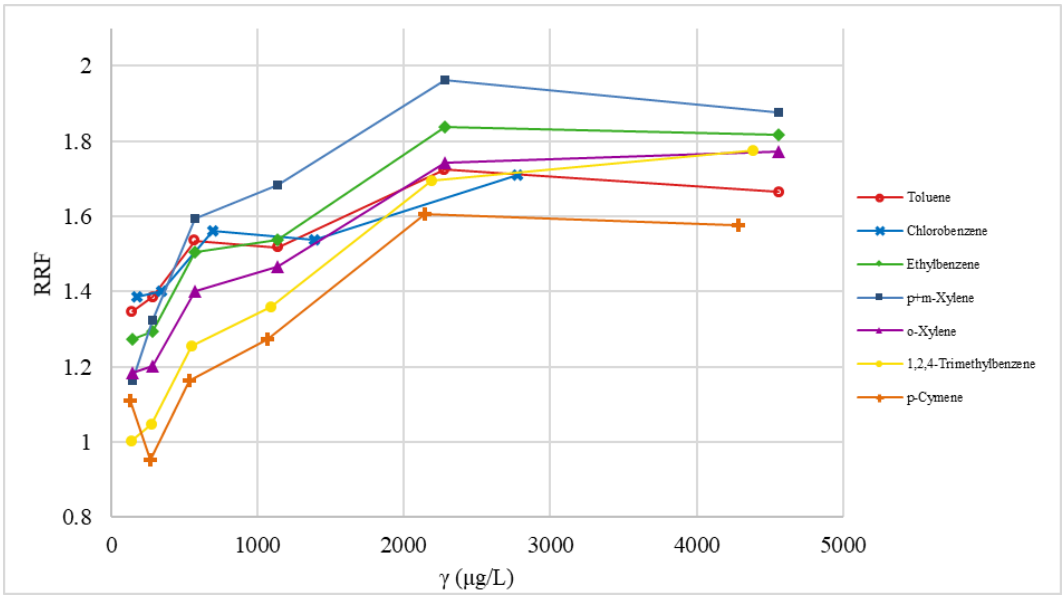
Component	LOD (μg/l)	LOQ (μg/l)	RSD (%)
Benzene	20.85	68.80	14.72
Toluene	2.47	7.50	5.24
Ethylbenzene	3.68	11.15	8.95
p+*m*-Xylene	2.23	6.75	6.50
o-Xylene	1.83	5.54	11.12
Trichloroethylene	1.19	3.61	5.71
Tetrachloroethylene	0.98	2.98	5.13
Butyl acetate	10.43	31.60	14.83
Chlorobenzene	6.25	18.95	5.32
α-Pinene	4.35	13.18	7.90
β-Pinene	2.73	8.26	8.37
1,2,4-Trimethylbenzene	2.32	7.02	8.11
p-Cymene	0.47	1.42	8.73
Limonene	2.65	8.04	9.76

3.2.3. *Relative response factors (RRF)*

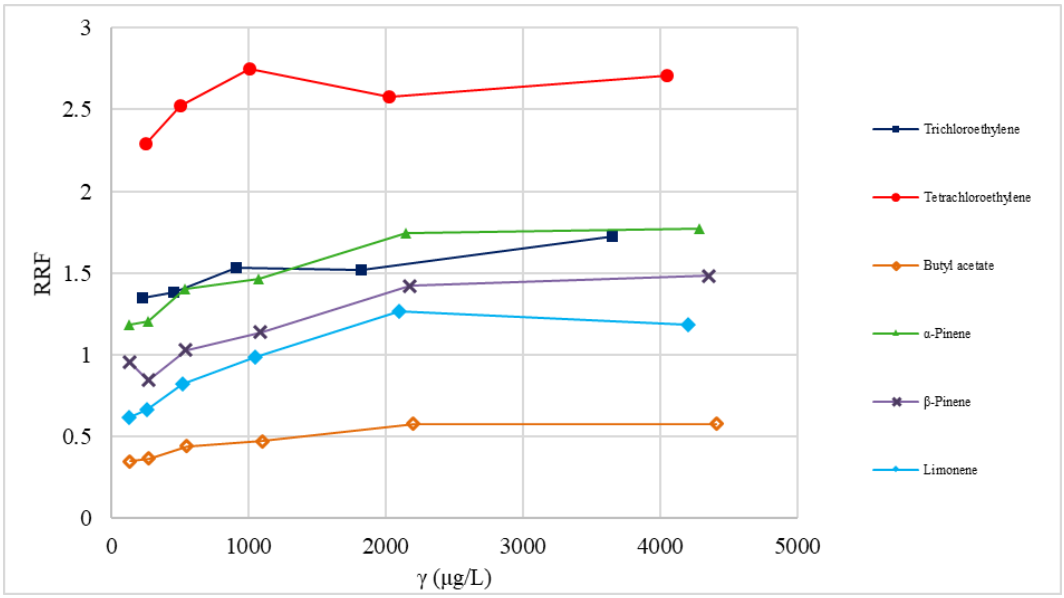
Moreover, the relative response factor for each analyte was calculated at each of the six concentration levels in relation to anisole (γ = 1990 µg/l), which served as the internal standard (all data in supplementary Table S2). For benzene, RRF values were calculated using peak heights instead of peak areas due to the previously mentioned reasons. As expected, the relative response factors (RRFs) exhibited a slight increase with rising concentration of the components in the standard solution, as illustrated in Figure 2.

The average values of RRF and their respective RSD for each target VOC across the studied concentration range are given in Table 4. All RSD values are below 30 % (except for benzene), which aligns with the common requirements in standard methods (RSD ≤ 30 %).[10] This indicates that quantification based on the average relative response factor of the detector for a given analyte can be applicable. In summary, the results support the use of anisole as an effective internal standard.

Toluene, chlorobenzene, and C_6-C_2- and C_6-C_3-substituted benzenes exhibited similar average RRFs, ranging from 1.280 to 1.601, with an RSD of 7.83 %.



a)



b)

Fig. 2. Dependence of RRFs on the analyte concentrations for the studied:
a) benzene derivatives and b) other studied VOCs listed in the respective figure

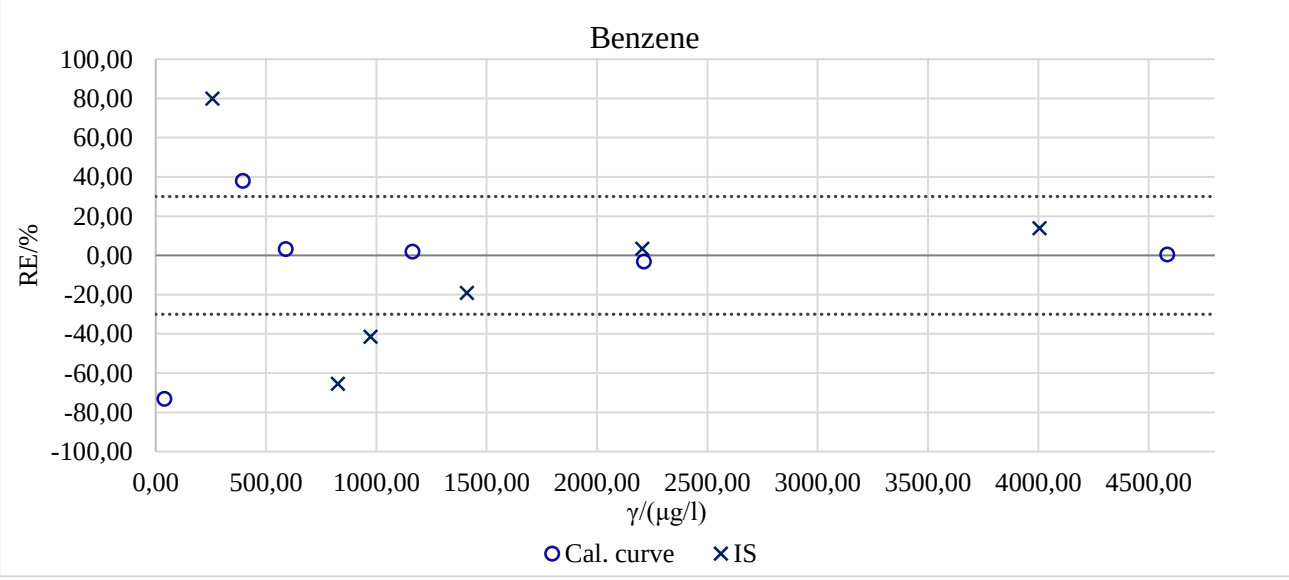
3.2.4. *Comparison between results of the two quantification methods*

To compare the results obtained from external calibration and internal standard quantification methods and evaluate their accuracy, standard solutions with known concentrations of all compounds were analyzed. The concentration of each compound in the test solutions was calculated using both approaches for each of the six concentration levels. The internal standard method employed the analyte's RRF values obtained from a standard solution with a medium concentration of 2000 μg/l, which is close to the concentration of the internal standard (1990 μg/l). The difference between the back-calculated concentration and the nominal concentration represents the error, quantitatively as the relative error (RE), calculated by dividing this difference by the nominal concentration. This parameter has been previously applied in the analytical literature to assess the error in quantification methods.[16,40] The back-calculated concentrations of each target VOC in the six standard solutions, along with the relative errors compared to the nominal mass concentration, are provided in supplementary Table S3. A visual representation of the relative errors obtained is given in Figure 3, with the dashed lines indicating the recommended limit for the acceptable values of the relative error within ± 30 %.[10]

As shown in Figure 3, both methods gave comparable results, with the calculated concentrations for most VOCs falling within ± 30% of the theoretical concentration. The only exceptions were the back-calculated concentration of butyl acetate in the lowest concentration solution using the internal standard quantification method, and the concentrations of benzene at the lowest concentration levels for both external calibration and internal standard methods, where the RE of the calculated concentration exceeded 30%.

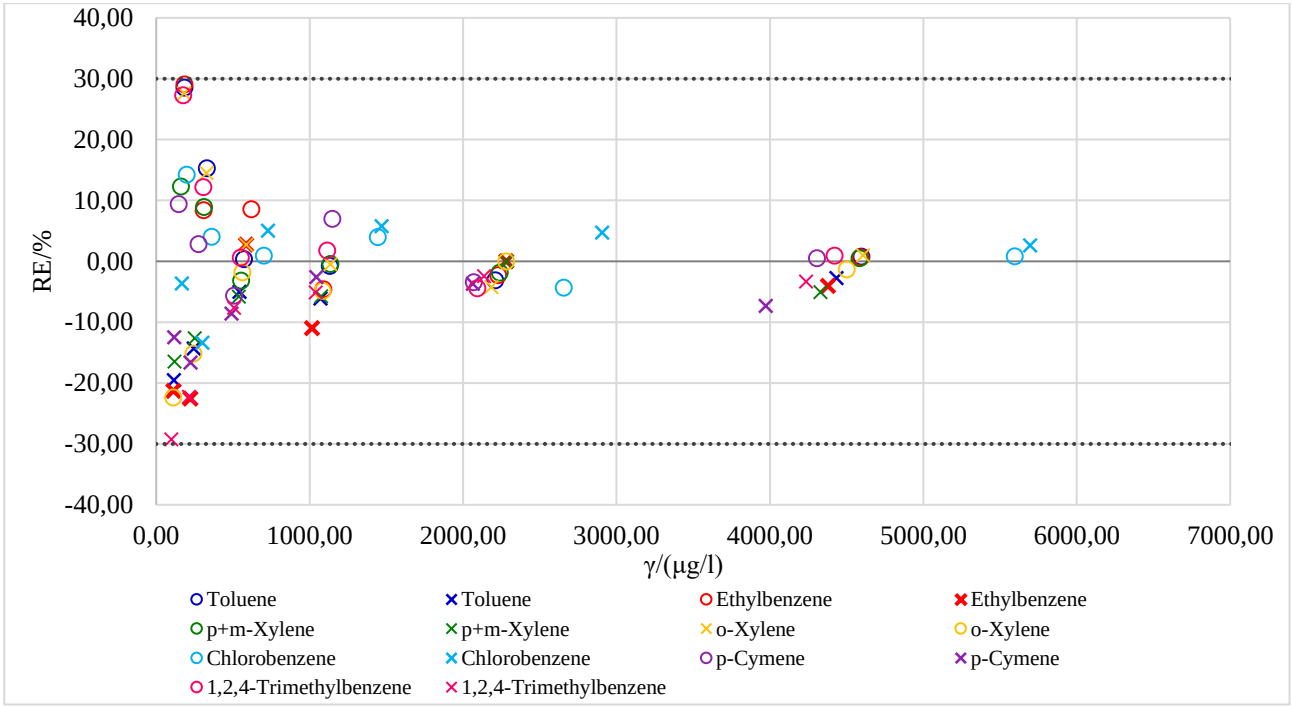
The observed differences among the target analytes did not suggest a pattern or trend for any compound; instead, the variations appear to be random. A general trend noted with both quantification methods was that the relative error tended to be greater at lower concentrations. It was demonstrated that the relative error decreased as the concentration of VOCs increased. In other words, at lower concentrations, even minor inconsistencies or errors in measurement can significantly affect the overall results, leading to increased variability. This phenomenon is commonly encountered in quantitative analysis, where precision and accuracy are more challenging at lower concentrations, necessitating higher allowable limits.

The differences in measurements across all compounds were comparable ($p > 0.05$, paired samples *t*-test, H_0: Mean = 0; see supplementary Table S4), indicating that there is no systematic bias in the quantification techniques. The mean difference between the two measurements was neither consistently positive nor negative. This demonstrates that the two approaches are comparable in terms of quantification performance within the examined concentration range.

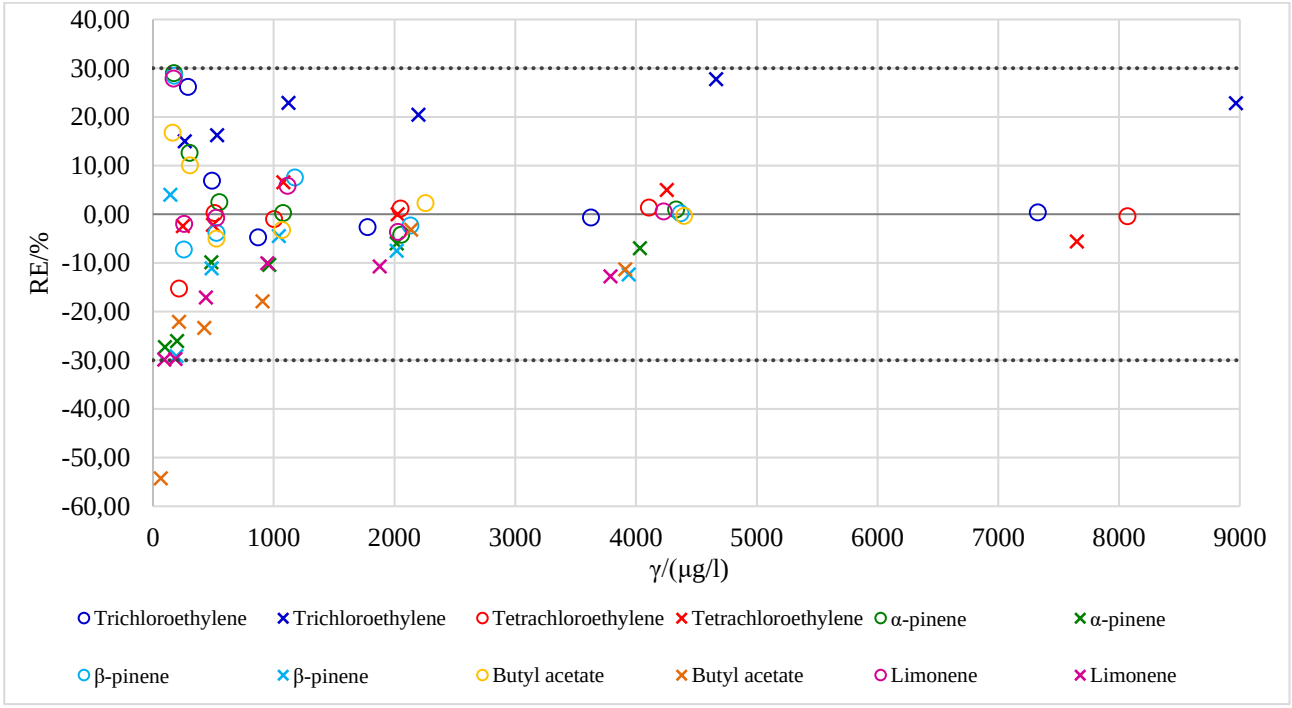


a)

Fig. 3. Relative errors of the back-calculated mass concentrations according to the external calibration curve method (dots) and internal standard method (crosses) with respect to the nominal mass concentration for the studied: a) benzene; b) benzene derivatives; and c) other studied VOCs (dashed lines indicate the recommended limit values for the relative error of ± 30 %)



b)



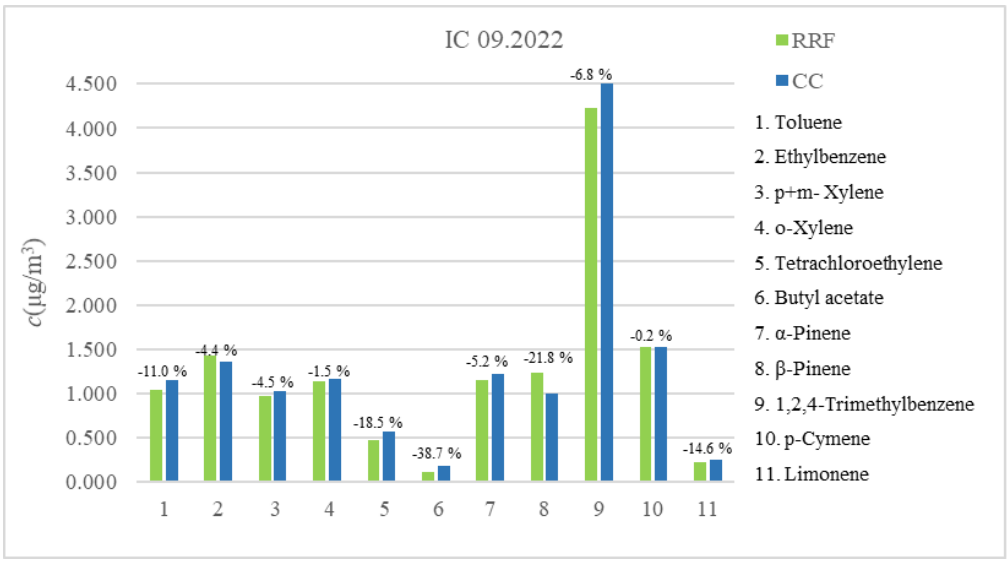
c)

Fig. 3. Relative errors of the back-calculated mass concentrations according to the external calibration curve method (dots) and internal standard method (crosses) with respect to the nominal mass concentration for the studied: a) benzene; b) benzene derivatives; and c) other studied VOCs (dashed lines indicate the recommended limit values for the relative error of ± 30 %)

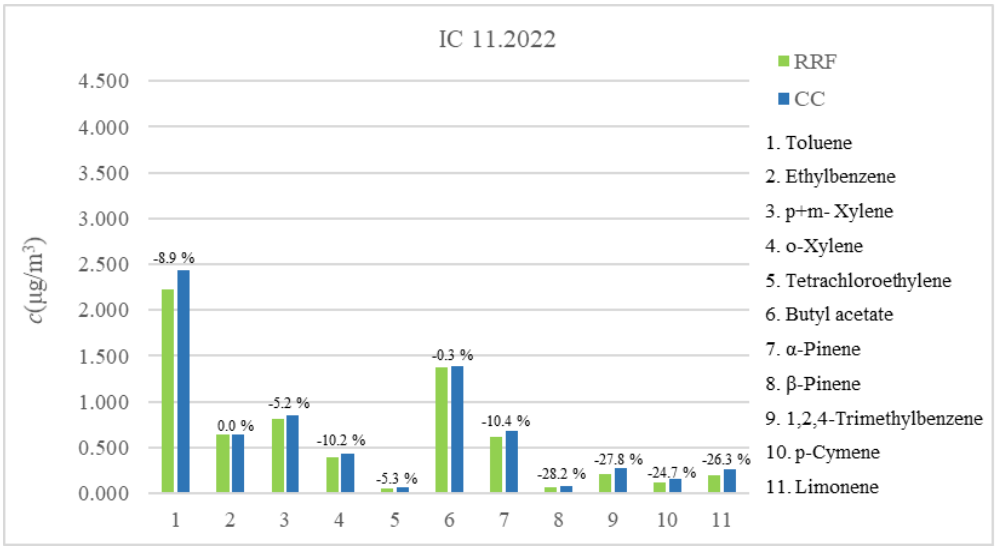
3.2.5. *Application of both quantification methods on real samples*

In the final step, in order to review the agreement between the results obtained from both quantification methods applied to analysis of real samples, air samples were collected from an urban location near the Institute of Chemistry in Skopje in September and November 2022. Details regarding sample preparation and calculations of the estimated air concentration are provided elsewhere.[34] Eleven compounds were detected and quantified using the

two quantification approaches. Numerical data for the calculated concentrations and the relative differences between the values obtained from the two quantification methods are given in the supplementary Table S5, with a graphical representation of these results provided in Figure 4. These findings further confirm the applicability of anisole as an internal standard for the quantification of VOCs in air samples following solvent desorption from adsorbents, demonstrating a more convenient approach compared to external calibration and construction of calibration curves for each analyte.



a)



b)

Fig. 4. Determined concentrations of VOCs using both internal standard method (IS) and calibration curve (CC) method with relative error in % with respect to CC for real air samples taken in: a) 09.2022; b) 11.2022. Location: Institute of Chemistry (IC), Skopje (42.00139° N, 21.45337° E).

4. CONCLUSION

Analyzing volatile organic compounds in air is a challenging task, as they are usually present at low concentrations (pg/l to µg/l) in ambient air. Quantifying these compounds using calibration curves for each substance can be time-consuming and susceptible to analytical inaccuracies. In this study, we compared two quantification approaches: one based on external calibration and the other utilizing anisole as an internal standard. The latter one was systematically evaluated for its reliability and applicability, as it is more practical and simpler for routine use. The results demonstrate that both approaches are comparable in terms of quantification performance across the examined concen-

tration range for all target analytes, except benzene, where the issue lies not with the quantification method but with separation, which can be addressed through optimization of GC parameters, such as by using pulsed splitless injection. In summary, these findings support the use of anisole as an internal standard for VOC quantification, offering a simple and reliable method that serves as a convenient alternative to external standard calibration by eliminating the need for separate calibration curves for each analyte, thereby saving time and resources.

Acknowledgements. This work was supported by the Ministry of Education and Science of RN Macedonia [grant number 15-15590/16, 2021] within the project for improving the capacity of the Laboratory for chromatographic analysis for environmental analyses.

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