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Analysis of volatile sulphur compounds in breath by gas chromatography–mass spectrometry using a three-stage cryogenic trapping preconcentration system

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Abstract

A method for the determination of trace volatile sulphur compounds (VSCs) including methanethiol, dimethyl sulfide (DMS) and dimethyl disulfide (DMDS) at low ppbv (volume/volume) in breath has been developed using a large volume preconcentration technique prior to capillary GC–MS analysis. The breath sample was collected in a 6-l fused-silica-lined stainless steel canister and introduced into the three-stage cryogenic trapping preconcentration system by GC–MS in the total ion monitoring (scan) mode. The water condensation effect of breath sample inside the canister, which is due to the difference between human body temperature and laboratory temperature, was examined. The condensed water in the fused-silica-lined canister at 24°C did not affect the recoveries of VSCs within 12 h. As this three-stage cryogenic trapping preconcentration technique made it possible to remove excess water {relative humidity (RH) >95%} and carbon dioxide (3.8%) without loss of the VSCs, more than 400 ml of the breath sample could be concentrated. The detection limits of methanethiol, DMS and DMDS in a breath sample using this method were 0.13, 0.09 and 0.15 ppbv, respectively. © 2001 Published by Elsevier Science B.V.

Keywords: Cryogenic; Trapping preconcentration; Volatile sulphur compounds

1. Introduction

Although volatile sulphur compounds (VSCs) are detected as key compounds of the malodorous in human breath, it is very difficult to collect, store and analyze them at trace levels because of their highly adsorptive, reactive and very volatile properties. In addition, there are huge matrices such as water

(RH>95%) and carbon dioxide (3.8%) in breath. In general, there are two approaches for the GC analysis of VSC in a gaseous sample. One is a direct injection of a sample into the GC using a sampling loop (0.5–5 ml) or gas tight syringe, the other is the enrichment of a large volume sample prior to the GC analysis. Tedlar bag sampling with the direct injection technique for the GC analysis of VSC has been widely used, however, the sensitivity is considered to be limited because of the small volume sampled in GC analysis with a sulphur selective detector such as FPD [1–3]. These methods were

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applied to the analysis of VSCs at sub-ppmv to ppmv level. In order to get a high sensitivity with the loop injection technique without the problem of matrix interference, capillary GC with a sulphur chemiluminescence detector (SCD) [4] or the selected ion monitoring (SIM) mode in GC–MS [5] were performed for the liquid petroleum gas (LPG) samples. The detection limits of VSCs by these methods were 5 ppbv for carbonyl sulfide [4], 1 ppmv for DMS and 10 ppmv for tert.-butanethiol, respectively [5]. In the only report at the time on the use of the loop injection technique for the analysis of ppbv level VSCs in breath, Blanchette obtained detection limit for methanethiol of 15 ppbv [6]. There are a variety of enrichment techniques for breath analysis such as thermal desorption GC–MS with a Tenax (2,6-diphenyl-*p*-phenylene oxide) adsorbent tube [7,8], and headspace GC–MS with Tenax [9]. However, these methods have not been suitable for very volatile compounds (in general, those with boiling points <60°C) because of the small break through volume on Tenax. Although whole column cryogenic trapping with a fused-silica capillary [10] and canister-based method using cryogenic trapping [11] have been used for volatile organic compounds (VOCs) with higher vapor pressure, they only focused on non-polar VOCs such as halogenated hydrocarbons and aromatic hydrocarbons. The maximum sample volume available for a breath sample was 100 ml because of interference by the huge amount of carbon dioxide [11]. In this paper, a method for determining ppbv levels of VSCs including methanethiol, DMS and DMDS in breath has been developed by using a large volume (400 ml) enrichment of a sample with a matrix control technique. VSCs in breath were collected in a fused-silica-lined canister and introduced into a three-stage cryogenic trapping preconcentration system followed by GC–MS analysis in the scan mode.

2. Experimental

2.1. Chemicals and preparation of standard gases

Methanethiol standard gas (100 ppmv) in a 10-l aluminum cylinder with nitrogen (10 MPa) was purchased from Sumitomo Seika Chemicals (Tokyo,

Japan). DMS and DMDS purchased from Tokyo Kasei Kogyo (Tokyo, Japan) were initially prepared at 100 ppmv each in a fused-silica lined canister with zero grade nitrogen (303 kPa). Then 100 ppmv of methanethiol, DMS and DMDS were dynamically diluted and mixed into a 6-l fused-silica-lined canister using an Entech 4620 dynamic dilution system (Entech Instruments Inc.) with humidified zero grade nitrogen. Concentrations of 1 ppmv per compound in the fused-silica-lined canister were obtained as stock standard gas mixtures. Ethanol, isopropanol, propanol, isobutanol, butanol, acetone, methyl ethyl ketone, methyl isobutyl ketone, ethyl acetate, butyl acetate, diethyl ether, methyl tert.-butyl ether, ethanethiol, propanethiol, sec-butanethiol, tert.-butanethiol and butanethiol were purchased from Wako Pure Chemicals (Osaka, Japan). These standards were initially prepared at 100 ppmv each in a fused-silica-lined canister with zero grade nitrogen (303 kPa). Then 100 ppmv of mixtures were dynamically diluted and mixed into a 6-l fused-silica-lined canister using an Entech 4620 dynamic dilution system with humidified zero grade nitrogen. Concentrations of 1 ppmv per compound in the fused-silica-lined canister were obtained as stock standard gas mixtures. The US EPA TO-14 method reference standard gas mixtures (41 compounds including acrylonitrile and 1,3-butadiene, 1 ppmv) in a 10-l aluminum cylinder with nitrogen (10 MPa) was purchased from Sumitomo Seika Chemicals. The stock standard gas mixtures and the US EPA TO-14 method reference standard gas mixtures were used for the recovery test with the dynamic dilution system shown in Fig. 1. Final standard gas mixtures for the recovery test had concentrations of 2.5–10 ppbv per compound in an RH 70% nitrogen. To eliminate the adsorption of the test mixtures onto the interior surface of the sample paths in the dynamic dilution system, a fused-silica-lined stainless steel tube was used for all the sample paths.

The working standard gas mixtures of VSCs were prepared at 1 to 100 ppbv by the static dilution system using humidified zero grade nitrogen.

2.2. Apparatus

The 6-l fused-silica-lined and SUMMA canisters were purchased from Entech Instruments Inc. The

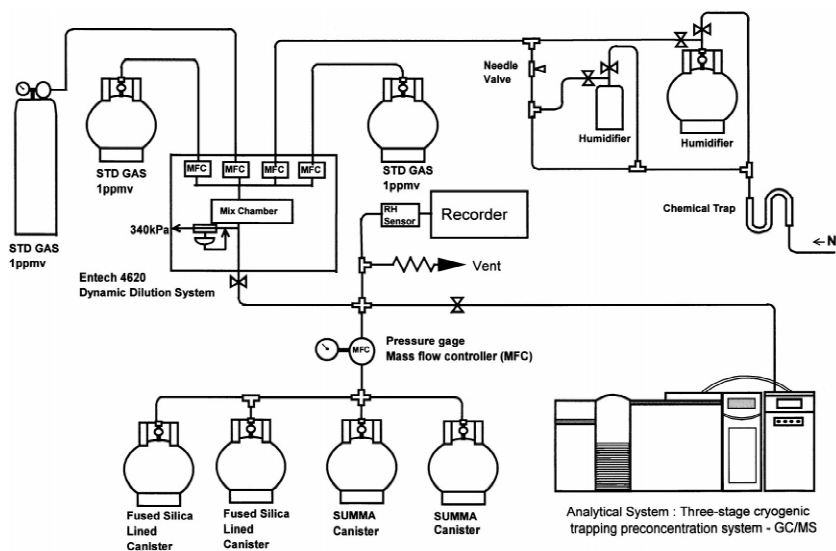


Fig. 1. Dynamic dilution system used to prepare test mixtures.

three-stage cryogenic trapping preconcentration and GC–MS analysis were performed using an Entech7100 preconcentrator and an Agilent 6890GC with a 5973 MSD from Agilent Technologies (CA, USA).

2.3. Sampling and preconcentration procedures

Several stainless steel canisters such as electro-polished, SUMMA polished, fused-silica-lined and multi-layer pretreated ones have been investigated to sample and store polar volatile organic compounds (VOCs) and VSCs [12–14]. The fused-silica-lined canister and the multi-layer pretreated canister that have a thinner, high-density fused-silica interior surface that showed good recovery for VSCs with any relative humidity in contrast to the traditional interior treated canisters such as electro-polished and SUMMA polished that have a metal surface [13]. A 6-l breath sample was collected in the fused-silica-lined canister via a quarter inch fused-silica-lined stainless steel tube (grab sampling). To eliminate the adsorption of the VSCs onto the interior surface of the sample paths in the three-stage cryogenic trapping preconcentration system, a fused-silica-lined stainless steel tube was also used for all the sample paths. After purging of the inlet line using high purity nitrogen, the breath sample was pumped from

the canister into the system at the flow-rate of 100 ml/min as shown in Fig. 2. The 400 ml of breath sample were first concentrated to about a 0.5-ml volume in a glass bead cryogenic trap (M1) at -150°C . The trap was then heated to 20°C and was held there while slowly passing helium through it to transfer these compounds to a secondary Tenax trap (M2) held at -30°C . After transfer to M2, the VSCs could be back-flushed while heating to be further focused on a capillary focusing trap for rapid injection onto the analytical column.

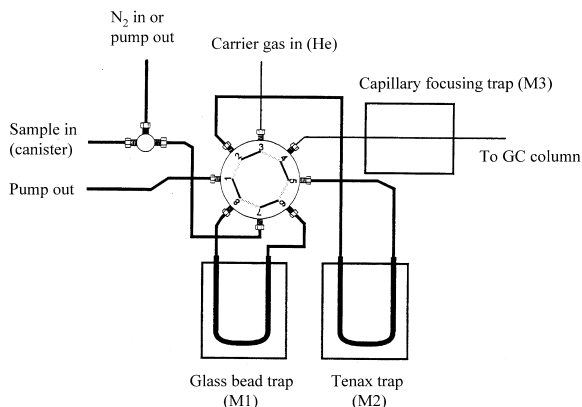


Fig. 2. Flow system for the preconcentration of VSCs from breath.

190 Table 1

191 Experimental conditions

193	Preconcentrator	Entech 7100
194	M1 (glass beads) trap temp.	–150°C
195	M1 (glass beads) purge temp.	20°C
196	M2 (Tenax) trap temp.	–30°C
197	M2 (Tenax) desorb temp.	180°C
198	M3 (capillary) trap temp.	–185°C
199	M3 (capillary) inj. temp.	75°C
200	Gas chromatograph	Agilent Technologies 6890
201	Column1	HP-1, 60 m length×0.32 mm I.D., 1.0 μm thickness
202	Column flow	1 ml/min constant flow mode
203	Oven temp.1	35°C (5 min)–5°C/min–220°C (5 min)
204	Mass spectrometer	Agilent Technologies 5973
205	Ionization mode	EI
206	Scan	m/z 29 to 300 in 0.45 s
207	Molecular ions used for determination	Methanethiol m/z 48, DMS m/z 62, DMDS m/z 94

209 2.4. GC–MS analysis

210 A HP-1 fused-silica capillary column (100%
 211 dimethylsilicone, 60 m length×0.32 mm I.D., 1.0
 212 μm film thickness, Agilent Technologies) was used.
 213 The oven temperature was programmed from 35°C
 214 for 5 min, ramped at 5°C/min to 80°C, then with a
 215 second ramp of 15°C/min to 220°C for 5 min. The
 216 helium carrier gas was operated at a rate of 1
 217 ml/min. The mass spectrometer was operated in the
 218 scan mode with the electron ionization (electron
 219 accelerating voltage: 70 V). The scan was set from
 220 m/z 29 to 300 in 0.45 s. For determination of the
 221 target compounds selected ion chromatograms over
 222 molecular ions (methanethiol: m/z 48, DMS: m/z 62
 223 and DMDS: m/z 94) were used. All the analytical
 224 conditions are shown in Table 1.

225 3. Results and discussion

226 3.1. Canister sampling

227 It is generally recognized that certain minimum
 228 levels of relative humidity are necessary to effect
 229 good recoveries of VOCs from the stainless steel
 230 canister [15]. However, the breath sample has very
 231 high humidity (RH>95%) and higher temperature
 232 than normal ambient air sample. The normal labora-
 233 tory temperature may cause condensation of liquid

234 water within the canister. The compound that has
 235 sufficiently large water solubility may be partially
 236 absorbed into the aqueous phase and may not be
 237 recovered. The amount of condensed water in the 6-l
 238 canister at 24°C (laboratory temperature), which is
 239 generated from the 6-l breath sample at 36°C (human
 240 body temperature), is calculated by the ideal gas
 241 equation. Taking values of 44.569 mmHg (760
 242 mmHg=101.3 kPa=1 atm) for saturated vapor
 243 pressure of water at 36°C, 22.375 mmHg for satu-
 244 rated vapor pressure of water at 24°C, 6 l for breath
 245 sample volume and 0.082 atm l/mol k for the gas
 246 constant, the moles of 6 l gaseous water which have
 247 saturated vapor pressure at 36°C and 24°C are
 248 calculated to be 0.01386 mol and 0.007242 mol,
 249 respectively. Consequently, the amount of condensed
 250 water in the 6-l canister at 24°C is calculated to be
 251 about 0.12 ml. We examined the effect of the
 252 condensed water in the canister for the recoveries of
 253 VSCs and VOCs from the high humidity sample.
 254 Evacuated 6-l fused-silica-lined canisters and 6-l
 255 SUMMA canisters were prepared by spiking 0.2 ml
 256 of water prior to the loading of the standard gas
 257 mixture. The test mixtures of standard gas, which has
 258 the concentration of 2.5–10 ppbv per compound for
 259 VOCs and VSCs in an RH 70% nitrogen, were
 260 introduced into the analytical system and the canis-
 261 ters (Fig. 1). After the test mixture analysis (pre-
 262 collection analysis), canisters were filled about to
 263 ambient pressure with the test mixture (RH 70%).

301 The amount of condensed water in each canister was
 302 calculated to be about 0.16 ml. Then the test mixture
 303 was reanalyzed (post-collection analysis). After 12 h
 304 equilibration, the samples were analyzed. The re-
 305 covery of the test mixture compounds from canisters
 306 was determined by comparing the mean of the
 307 measured values to the mean of the pre- and post-
 308 collection analyses from the dynamic dilution sys-
 309 tem. For the fused-silica-lined canisters, the re-
 310 coveries ranged from 90 to 105% for halogenated
 311 hydrocarbons, aromatic hydrocarbons and 1,3-
 312 butadiene, 86 to 102% for oxygenated hydrocarbons
 313 except all alcohol, 97 to 102% for VSCs and 36 to
 314 54% for alcohol. For the SUMMA canisters, the
 315 recoveries ranged from 96 to 109% for halogenated
 316 hydrocarbons, aromatic hydrocarbons and 1,3-
 317 butadiene, 82 to 104% for oxygenated hydrocarbons
 318 except all alcohol, 90 to 97% for VSCs except all
 319 thiols and 42 to 76% for alcohol. All thiols were not
 320 recovered from SUMMA canisters. This is mainly
 321 due to adsorption onto the interior surface because
 322 the major difference between the SUMMA canister
 323 and the fused-silica-lined canister is the material of
 324 the interior surface. The SUMMA canister has a
 325 nickel-chromium oxide interior surface in contrast to
 326 the fused-silica-lined canister that has a thinner,
 327 high-density fused-silica interior surface. Thus the
 328 main drawbacks of canister sampling for thiol analy-
 329 sis using the SUMMA canister is the adsorption onto
 330 the metal interior surface rather than the effect of
 331 condensed water. Using the fused-silica-lined canis-
 332 ter, about 0.16 ml of condensed water did not affect
 333 the recoveries of test mixture compounds except
 334 alcohol within 12 h. The recoveries of the test
 335 mixture compounds from the canisters and the
 336 Henry's law constants (k_H) [16–22] are shown in
 337 Table 2.

338 3.2. Preconcentration

339 The scan mode in the GC–MS analysis is useful to
 340 determine target compounds with any other sample
 341 matrix. However, in order to get enough sensitivity
 342 for the ppbv level analysis of VSCs in the scan
 343 mode, the preconcentration volume of the sample
 344 must be more than 100 ml. More than 100 ml of the
 345 breath sample preconcentration without water and
 346 carbon dioxide management will cause clogging of

Table 2
 The recoveries of the test mixture compounds from the canisters
 and Henry's law constants (k_H)

Compound	Recovery (%)		k_H^a (mol/atm)
	FSL ^b	SUMMA ^c	
Dichloromethane	96	96	0.39 [16]
Chloroform	98	100	0.27 [16]
1,2-Dichloroethane	96	103	0.92 [16]
Benzene	97	102	0.18 [16]
Toluene	93	108	0.15 [16]
1,3-Butadiene	98	102	0.014 [16]
Ethanol	54	42	190 [17]
Isopropanol	38	56	130 [17]
Isobutanol	43	76	110 [17]
Acetone	86	82	26 [17]
Methyl ethyl ketone	98	98	18 [17]
Methyl isobutyl ketone	99	99	2.2 [18]
Ethyl acetate	97	96	6.5 [19]
Butyl acetate	102	104	3.5 [19]
Diethyl ether	98	92	1.2 [20]
Methyl t-butyl ether	100	100	1.6 [21]
Acrylonitrile	94	94	7.3 [19]
Methanethiol	97	ND ^d	0.39 [22]
Ethanethiol	99	ND	0.28 [22]
Propanethiol	101	ND	0.25 [22]
Butanethiol	102	ND	0.22 [22]

The test mixtures have a concentration of 2.5–10 ppbv per
 compound.

^a k_H = Henry's law constants.

^b FSL, fused-silica-lined canister.

^c SUMMA, SUMMA canister.

^d ND, not detected.

the cryogenic trap [10], or injection of a huge matrix
 into the GC–MS. The analytes that co-elute with
 these matrices will produce a serious problem such
 as poor peak shape and sensitivity suppression. A
 three-stage cryogenic preconcentration technique has
 been developed to analyze VOCs in humid air with a
 huge matrix management [23]. This made possible
 the removal of excess water and carbon dioxide from
 the ambient air sample without loss of the VOCs and
 polar VOCs. It is analogous to the purge and trap
 (P&T) used in water analysis, only on a much
 smaller scale between the cryogenic glass beads and
 Tenax traps. As the vapor pressures of the VOCs and
 water are roughly the same level at less than ambient
 temperature, the temperature during the purge pro-
 cess of a glass bead trap is set at ambient tempera-
 ture. The vaporization rate of the VOCs and water
 are of the same order of magnitude even though total

382 amount is significantly different. Therefore, water is
 383 left in the glass bead trap held at ambient tempera-
 384 ture, VOCs and carbon dioxide are easily purged by
 385 helium gas. One hundred ml of an ambient air
 386 sample would only yield less than 2 μl of water
 387 rather than the 5000 μl used in the water analysis.
 388 The distribution of the condensed water on the glass
 389 beads in the trap should further facilitate the transfer
 390 of VOCs and polar VOCs to the gas phase. Finally,
 391 the VOCs are trapped in the Tenax trap, while carbon
 392 dioxide breaks through the Tenax trap. Several
 393 parameters such as purge temperature, purge flow,
 394 purge volume and trap temperature on the recoveries
 395 and peak shapes of the VOCs were optimized with
 396 ambient air matrix [24]. Since the vapor pressures of
 397 the DMDS (DMDS has a lower volatility in the
 398 target VSCs) and water are almost the same at 20°C
 399 (3 kPa and 2 kPa, respectively), the purge tempera-
 400 ture of 20°C was chosen. To make carbon dioxide
 401 (b.p. -78°C) pass through the Tenax trap without
 402 break through of the methanethiol (b.p. -6°C), the
 403 purge flow and trap temperature were set at 10
 404 ml/min and -30°C , respectively [24]. The major
 405 differences between the ambient air and breath
 406 sample matrix are the amount of water (RH 95%)
 407 and carbon dioxide (3.8%). As the key parameter of
 408 the three-stage cryogenic trapping to eliminate these
 409 matrices in the breath sample is the purge volume,
 410 the purge volume during the preconcentration was
 411 optimized with the matrix-spiked VSCs standard gas
 412 mixture (400 ml samples, 40 ppbv each) in the
 413 canisters. Fig. 3 shows the influence of the purge
 414 volume on the purge efficiency of the VSCs with a
 415 high humidity (RH 100%). Only the purge volume
 416 of 10 ml was enough to reach the maximum yield
 417 and there was no degradation in the yield for the
 418 purge volume of 120 ml without effects by the water
 419 matrix on the GC–MS analysis. Fig. 4 shows the
 420 same influence under the same conditions except for
 421 a matrix spiking of 3.8% carbon dioxide. It can be
 422 seen that there were no responses of any of the VSCs
 423 between the purge volume of 1 ml and 5 ml in
 424 contrast with the carbon dioxide responses (plotted
 425 with 1/300 Y-axis scale). Carbon dioxide responses
 426 were dramatically decreased between the purge
 427 volumes of 5 and 20 ml. The peaks of methanethiol
 428 and DMS were still very small and broad at the
 429 purge volume of 10 ml (Fig. 5). As less than a 20-ml

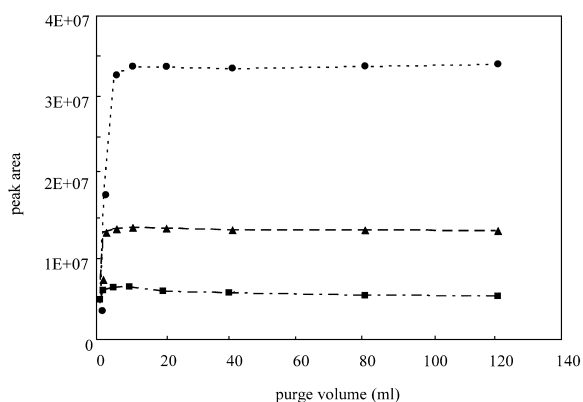


Fig. 3. Influence of purge volume on the purge efficiency with high humidity (RH 100%) matrix: ■, methanethiol; ▲, DMS; ●, DMDS.

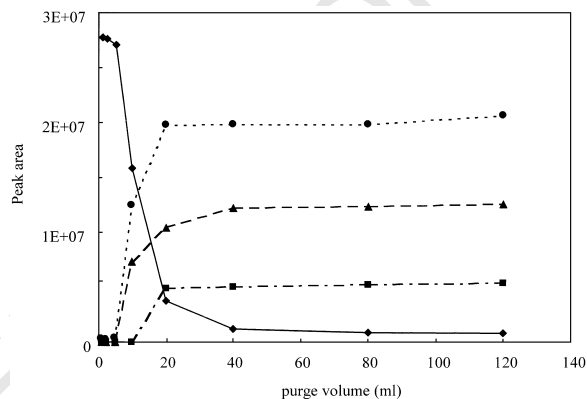


Fig. 4. Influence of purge volume on the purge efficiency with high humidity (RH 100%) and 3.8% carbon dioxide matrices: ♦, CO₂; ■, methanethiol; ▲, DMS; ●, DMDS.

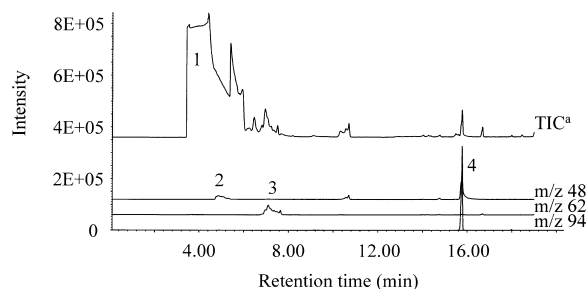
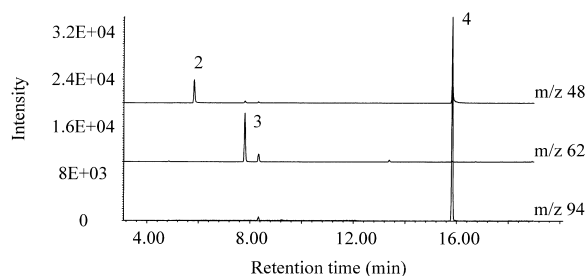
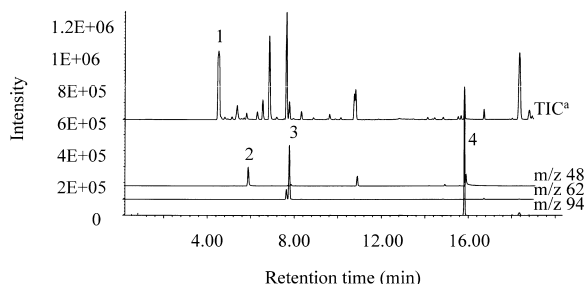


Fig. 5. The influence of carbon dioxide for VSC analysis with MPT using 10 ml of purge volume. (1) CO₂, (2) methanethiol, (3) DMS, (4) DMDS. Concentration of VSCs STD: 40 ppbv each. ^aTIC is multiplied by 0.1.



432

433 Fig. 6. Removal of carbon dioxide for VSC analysis with MPT
 434 using 80 ml of purge volume. (1) CO₂, (2) methanethiol, (3)
 435 DMS, (4) DMDS. Concentration of VSCs STD: 40 ppbv each.
 436 ^aTIC is multiplied by 0.1.

453 purge volume was not enough to remove the excess
 454 carbon dioxide from the secondary Tenax trap, a
 455 huge amount of carbon dioxide was introduced into
 456 GC–MS and caused signal suppression and serious
 457 chromatographic problems for methanethiol and
 458 DMS, which are early eluting VSCs. For DMDS,
 459 which has a lower volatility in the target VSCs, the
 460 purge efficiency was strongly affected by the huge
 461 amount of carbon dioxide on the glass bead trap.
 462 Consequently, more than 20 ml of purge volume was
 463 essential for the transfer of DMDS to the Tenax trap.
 464 The purge volume of 80 ml was chosen for further
 465 work. The total ion chromatograms (TIC) and mass
 466 chromatograms of the VSCs with huge matrices at
 467 purge volumes of 10 and 80 ml are shown in Figs. 5
 468 and 6, respectively.

469 3.3. Method validation and determination of VSCs 470 in breath

471 In order to validate the method, an ambient air
 472 sample (not including target VSCs) collected in a
 473 fused-silica-lined canister was prepared by spiking

450
 451 Fig. 7. Mass chromatograms of VSCs STD at 1 ppbv each. (2)
 452 Methanethiol, (3) DMS, (4) DMDS.

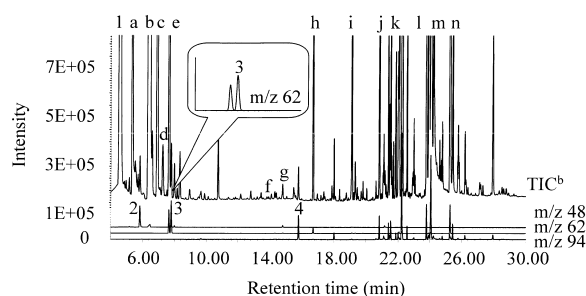
474 with VSCs and matrices such as high humidity (RH
 475 100%) and carbon dioxide (3.8%) prior to the
 476 sample collection. The linearity, sensitivity and
 477 recovery of the method were tested and are shown in
 478 Table 3. The seven points for the calibration curves
 479 for the VSCs were linear over a range 1 to 100 ppbv
 480 (1, 2, 5, 10, 20, 50, 100 ppbv) with correlation
 481 coefficients better than 0.9988 and a relative standard
 482 deviation (% RSD) of response factors better than
 483 7.4%. The mean recoveries of the VSCs at 10 ppbv
 484 within a day (24 h) were 83% (RSD 6.7%, $n=6$) for
 485 methanethiol, 98% (RSD 5.7%, $n=6$) for DMS and
 486 88% (RSD 11%, $n=6$) for DMDS, respectively. By
 487 using these calibration curves, the replicate analysis
 488 of the lowest level (1 ppbv, $n=6$) and three times
 489 the standard deviation (3 SD) of these amounts, the
 490 detection limits of methanethiol, DMS and DMDS
 491 were calculated to be 0.13, 0.09 and 0.15 ppbv,
 492 respectively. Mass chromatograms of the VSCs at 1
 493 ppbv are shown in Fig. 7. The developed method
 494 was then used for the breath sample. The 6-l breath
 495 samples ($n=6$) were collected from an examinee
 496 after using typical mouthwash solution that included
 497 a high ppm level of ethanol and flavor compounds
 498 such as menthol and mono-terpene (the examinee
 499 rinsed his mouth with 10 ml of mouthwash solution

437 Table 3

438 Method validation: correlation coefficients, detection limits and recoveries of VSCs

440 Compound	441 Correlation coefficient 442 r^2 (1–100 ppbv)	443 Detection limit 444 ppbv	445 Mean recovery 446 % ($n=6$)
443 Methanethiol	0.9999	0.13	83 (RSD 6.7%)
444 DMS	0.9991	0.09	98 (RSD 5.7%)
445 DMDS	0.9988	0.15	88 (RSD 11%)

447 Detection limits were calculated by the replicate analysis of 1 ppbv ($n=6$) and three times the standard deviation (3 SD) of these
 448 amounts. The mean recoveries within a day (24 h) were examined by measuring spiked sample at 10 ppbv.



502

503 Fig. 8. Example of determination of VSCs in breath. (1) CO₂, (2)
 504 methanethiol, (3) DMS, (4) DMDS, (a) acetaldehyde, (b) ethanol,
 505 (c) acetone, (d) IPA, (e) isoprene, (f) allyl methyl sulfide, (g)
 506 methyl propyl sulfide, (h) toluene, (i) allyl isothiocyanate, (j)
 507 α-pinene, (k) sabinene, (l) menthone, (m) menthol, (n) anethol.
 508 ^bTIC is multiplied by 0.5.

509 for 20 s). A 400-ml breath sample was used for the
 510 analysis from the 6-l canisters. Typical chromatograms of the breath sample are shown in Fig. 8.
 511 Well-defined mass chromatograms of the VSCs were
 512 obtained without interference from carbon dioxide
 513 and the other matrix compounds. The concentrations
 514 of methanethiol, DMS and DMDS were calculated as
 515 9.0 ± 0.7 , 5.4 ± 0.4 and 1.1 ± 0.1 ppbv, respectively
 516 (mean \pm SD, $n=6$). Satisfactory reproducibilities
 517 were obtained for all the VSCs with an RSD value
 518 ($n=6$) for the peak areas of the mass chromatograms
 519 used for determination between 5.1 and 7.6%. The
 520 breath samples were also found to contain other
 521 VSCs such as allylmethyl sulfide, methylpropyl
 522 sulfide, allylisothiocyanate and other miscellaneous
 523 compounds.
 524

525 4. Conclusion

526 A method for the determination of trace VSCs
 527 including methanethiol, DMS and DMDS at low
 528 ppbv in breath was developed. Although the water
 529 condensation of breath sample inside the canister
 530 occurred, the fused-silica-lined canister could be
 531 applied to the sampling of VSCs in breath. The
 532 combination technique of a fused-silica-lined canister
 533 and a three-stage cryogenic trapping system for
 534 sampling and preconcentration enabled a large vol-
 535 ume injection of the breath sample into the GC–MS
 536 without loss of the VSCs. By the optimization of the
 537 purge volume of three-stage cryogenic preconcen-

538 tration, the interference of huge matrices in breath
 539 such as high humidity (RH >95%) and carbon
 540 dioxide (3.8%) was eliminated and more than 400 ml
 541 of breath sample could be concentrated. The de-
 542 tection limits of methanethiol, DMS and DMDS in
 543 breath using this method were 0.13, 0.09 and 0.15
 544 ppbv, respectively. The detection limit of
 545 methanethiol was more than 100 times of the known
 546 method [6]. The method could successfully be
 547 applied to the analysis of VSCs at low ppbv levels in
 548 human breath. Furthermore, it was found that the
 549 breath samples contain allylmethyl sulfide,
 550 methylpropyl sulfide and allylisothiocyanate.

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