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Combination of accelerated solvent extraction and vortex-assisted liquid–liquid microextraction for the determination of dimethyl fumarate in textiles and leathers by gas chromatography–mass spectrometry

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ABSTRACT

A simple and environmentally friendly sample preparation procedure coupled with gas chromatography–mass spectrometry was developed to assay dimethyl fumarate in textiles and leathers. The sample preparation procedure involved an accelerated solvent extraction (ASE) using water as the extract solvent, followed by the extraction and concentration of dimethyl fumarate from the aqueous solution using vortex-assisted liquid–liquid microextraction (VALLME). The parameters affecting the ASE and VALLME were optimized to achieve the maximum extraction efficiency, and the performance of the developed method was evaluated. Good linearity was observed over the range assayed (0.01–1 mg/kg) with a regression coefficient of 0.998. The limit of detection and enrichment factor for the VALLME step were 0.001 mg/kg and 53, respectively. The intra- and inter-day precision were below 8.9%, and the recovery was approximately 84–103%. The as-developed method was successfully applied to textiles and leather samples.

1. Introduction

Dimethyl fumarate (DMFu, CAS Registry Number 624-49-7) is a white crystal that is a known inhibitor of mold growth with antibacterial properties [1]. Many cases of contact dermatitis related to consumer products that contain DMFu as an anti-mold agent have been reported throughout the European Union (EU) including France, Finland, Italy, Spain and the United Kingdom [2–7]. Thus, DMFu has been deemed an allergic sensitizer at low concentrations, causing eczema that is difficult to treat. On 17 March 2009, the European Commission decided to ban the use of this antifungal agent in all consumer products [8]. According to this decision, the quantity of DMFu in products should not exceed a maximum limit of 0.1 mg/kg of product or part of the product. This quantity is considered sufficiently below the 1 mg/kg concentration that caused a strong reaction in the aforementioned patch test. Therefore, the analytical method for evaluating DMFu should be able to reliably quantify DMFu at the parts-per-million (milligrams-per-kilogram) level.

Gas chromatography (GC) using an electron capture detector (ECD) or mass detector (MS) and high performance liquid chromatography (HPLC) are the methods typically employed to detect DMFu. Prior to analysis, a fractionation step is required due to the low DMFu level and the complexity of the matrices. Until now, few methods for determining DMFu in desiccants, anti-mold sachets and consumer products have been published [1,9–13], most of which are based on an ultrasonication extraction. Methanol, acetone and ethyl acetate are the solvents most commonly used for this purpose. However, they proved insufficient for the analysis of some manufactured materials, in particular leather products, due to the large quantity of compounds co-extracted from the sample matrices with organic solvents. Therefore, these extractions frequently required a post-extraction cleanup process [1,11,13]. Moreover, these methods require considerable solvent volumes and often require a preconcentration step to increase the detection sensitivity. Multiple sample preparation steps and a time-consuming solvent evaporation step can introduce errors in the results.

In recent years, accelerated solvent extraction (ASE) has widely been recognized for its ability to achieve recoveries comparable to those obtained with traditional methods. The ASE technique offers numerous advantages: it is rapid, requires low solvent volumes and allows the use of solvents with a wide range of polarities.

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In addition, the high pressure and temperature conditions enable a better penetration of the solvent into the matrix, which aids in the breaking of the intermolecular bonds [14]. Water is an appealing choice as an extraction medium because it is inexpensive and non-toxic. However, the properties of water are significantly altered at elevated temperatures and pressures, which leads to a dramatic increase in the solubility of less polar compounds.

Because ASE extracts are relatively dilute aqueous solutions, a subsequent preconcentration technique is required. Vortex-assisted liquid–liquid microextraction (VALLME), first developed by Yiantzi and co-workers [15], has the potential to simultaneously extract, clean and concentrate aqueous samples and can be used as an interface between ASE and gas chromatography. In VALLME, a microliter of extraction solvent is dispersed into an aqueous sample via vortex mixing. A fine liquid–liquid dispersion system is formed during the vortex process, and the mass transfer of the target analyte from the aqueous layer to the extraction solvent is facilitated due to the reduced diffusion distance and increased interfacial area [16]. This technique has attracted much attention and has been successfully used for the determination of polychlorinated biphenyls [17], phthalate esters [18], organophosphate pesticides [19] and ultraviolet filters [20] in water samples or wines.

This work combines ASE and VALLME to develop a VALLME technique for analyzing textiles and leather samples. The aim of present study is to develop, optimize and validate a new effective, simple, sensitive, and environmental friendly method that requires little consumption of toxic organic solvents.

2. Experimental

2.1. Chemicals and materials

The DMFu (99.0% purity) and internal standard naphthalene-d8 (98.3% purity) were supplied by Dr. Ehrenstorfer, GmbH (Augsburg, German). A 1000 mg/L DMFu stock solution was prepared in acetonitrile and stored at 4 °C. From the stock solution, intermediate and working standard solutions were prepared by diluting with deionized ultrapure water or trichloroethylene. The acetonitrile (HPLC grade) was purchased from TEDIA (Fairfield, USA), all other organic solvents (acetone, ethyl acetate, trichloroethylene, perchloroethylene and carbon tetrachloride) were of analytic grade and from Huadong Pharmaceutical Co., Ltd. (Hangzhou, China).

2.2. Instrumentation

The GC–MS analyses were performed in a selected ion monitoring (SIM) mode using a Thermo TRACE GC Ultra gas chromatograph (Milan, Italy) interfaced to a Thermo TRACE DSQII mass spectrometer (70 eV, electron impact mode) (Austin, USA) equipped with an automatic liquid sampler system. The chromatographic conditions were as follows: 30 m DB-5MS capillary column with a 0.25 mm i.d. and a 0.25 μm film thickness. Helium (99.999%) was used as the carrier gas at a constant velocity of 1.0 mL/min. The temperatures for the injector, MS transfer line and ion source were 250 °C, 280 °C and 250 °C, respectively. The oven temperature program was as follows: 50 °C held for 1 min, increased to 150 °C at 10 °C/min, held for 3 min, and ramped to 270 °C at 20 °C/min. The injection volume was 1 μL. Samples were introduced in splitless mode. The MS detector was operated in selected ion monitoring (SIM) mode, with ions of m/z 113, 114, 85 used for the peak-identification and m/z 113 for quantification.

The HPLC system consisted of a Waters 2695 separation module and 2996 photodiode array detector (Waters, USA). A Waters Symmetry® C18 (250 mm × 4.6 mm i.d., particle size = 5 μm) column was used. The operating temperature of the column was set at 30 °C. The chromatographic analysis was carried out using an isocratic elution of acetonitrile-water (30:70, v/v) as the mobile phase. The flow rate was 1.0 mL/min, and the eluate was monitored using UV detection at 216 nm.

An XK95-B vortex agitator (Jiangsu, China) and an Anke TDL 80-2B centrifuge (Shanghai, China) were utilized for vortex and centrifugation.

2.3. Accelerated solvent extraction and vortex-assisted liquid–liquid microextraction

The accelerated solvent extraction was performed using a Thermo Scientific ASE 350 Accelerated Solvent Extraction system (Sunnyvale, CA, USA) with 22 mL stainless steel extraction cells. Under the optimized conditions, a 2 g textile or leather sample was extracted with water at 40 °C and 1500 psi for one cycle of 10 min; the flush volume was set at 0%, and the solvent saver was enabled. After pressurization, the cell was purged with N_2 for 80 s, and the extract was collected in pre-cleaned glass vials. Finally, the extract was diluted to 25 mL and filtered with a 0.45 μm nylon filter.

As described in previous studies [15,16], a 5 mL aliquot of the aqueous extract was then placed in a 10 mL screw-cap glass centrifuge tube with a conical bottom, and 50 μL trichloroethylene was added. The mixture was vortexed at 2800 rpm for 2 min, then centrifuged for 5 min at 4000 rpm (maximum speed). The trichloroethylene phase was deposited at the bottom of the centrifuge tube, 20 μL of the sediment phase was transferred to a small sample vial using a microsyringe, and 2 μL naphthalene-d8 (10 μg/mL) was added as an internal standard. The sample was analyzed via GC–MS.

2.4. Calculations

The proposed ASE–VALLME method involves two processes: the extraction of DMFu from the textile/leather into water and then the extraction of DMFu from the extractant water to the organic phase, they were not exhaustive extraction. In addition, the VALLME technique is a combination of extraction and enrichment due to the high phase ratio of the donor (aqueous extractant) and the acceptor (extraction solvent).The extraction recovery (ER%) and enrichment factor (EF) were used to assess the performance of given procedure. The ER% was calculated according to the following equation:

$$\mathrm{ER}\ (\%) = 100 \times n_{\mathrm{found}}/n_0$$

in which n_{found} was the analyte amount obtained from the ASE or the overall ASE–VALLME method after addition of a known amount of the standard solution into the real sample, n_0 was the initial amount of the standard solution spiked to the real sample.

The enrichment factor (EF), defined as the ratio between the analyte concentration after preconcentration (C_{sed}) and the initial analyte concentration (C_0), can be calculated using the following equation:

$$\mathrm{EF} = C_{\mathrm{sed}}/C_0.$$

3. Results and discussion

3.1. Optimization of the ASE parameters

To optimize the ASE procedure, the critical parameters are likely to be the extraction solvent, the temperature, the static time and the number of extraction cycles. The extraction efficiency was

calculated via the recovery. Recovery extractions were carried out in triplicate using 2 g of a textile sample spiked at 10 mg/kg.

The temperature is the most important parameter in an ASE extraction. The extraction temperature influences the extraction kinetics and the solvent viscosities, therefore affecting the extraction efficiencies and overall recoveries [21]. This effect was studied at different temperatures ranging from 40 °C (the minimum controllable temperature of the ASE350 extractor) to 100 °C. The extraction experiments were conducted using water as the extraction solvent. The extracts were directly analyzed via HPLC. The results showed that extraction recovery for DMFu decreased when temperature was raised from 40 to 100 °C, varied from 91.0% to 53.9%. The highest extraction efficiency was obtained at 40 °C, this may be due to the fact that DMFu was partly lost by decomposition or volatilization at high temperature. Consequently, 40 °C was chosen as the optimal extraction temperature.

Increasing the static time can allow the compounds to diffuse into the extraction solvent. To evaluate whether the extraction time affects the extract recovery, triplicate extractions were performed with static times of 5, 7, 10, 12 and 15 min applying 1 cycle, at 40 °C. The recovery significantly improved by increasing the static time from 5 to 10 min. However, when static time was above 10 min, a slight decrease was observed. A static time of 10 min was selected because it provided good extraction efficiency with short analysis time. Then, the number of extraction cycles was varied from one to three. The second extraction cycle yielded approximately 9.1% of the extract quantity obtained in the first cycle, whereas the third extraction cycle yielded less than 1% of the extract obtained in the first cycle. Increasing the number of extraction cycles allowed more fresh solvent to pass through the sample but also increased the final volume of the extract. Thus, one cycle was selected to minimize the extract volume and analysis time. Flush percentage refers to the amount of solvent flushed through the cell following the static heating step, expressed as a percentage of the cell volume. Increasing the flush volume allowed more solvent to pass through the sample, but also increased the final volume of the extract. Extraction efficiencies of the analyte decreased with increasing flush volumes from 0% to 60%, Therefore, the flush volume was set at 0%.

3.2. Optimization of the VALLME procedure

The extraction solvent selection is critical to VALLME in this method. In general, an extraction solvent ideal for VALLME procedures should possess a higher density than water, low water solubility, and high extraction efficiency for the compounds of interest with good chromatographic behavior. Chloroform, trichloroethylene, perchloroethylene and carbon tetrachloride were investigated to determine the most suitable solvent for the extraction. An extraction solvent volume of 100 μL was used to extract a 5 mL aqueous DMFu standard solution at a concentration of 0.1 mg/L. Comparison of the enrichment factor obtained with the different extraction solvents showed that trichloroethylene is the most effective extraction solvent. Consequently, trichloroethylene was selected as the optimum extraction solvent and used in subsequent studies.

The quantity of solvent is another critical factor for highly enriching the analyte. The effect of the trichloroethylene volume was studied in the range of 50–100 μL. The results demonstrated that decreasing extraction solvent volume enhanced the performance of the microextraction process. The maximum EF was obtained at a 50 μL volume of trichloroethylene. The use of less extraction solvent results in smaller volume of the sediment phase and thus enhances the concentration ratio of analyte. In addition, the effect of using 30 and 40 μL of extraction solvent was investigated to obtain a higher enrichment factor. However, the sediment phase became unstable and its volume was insufficient for automatic injection. To ensure sufficient volume in the post-extraction phase and improved reproducibility for the subsequent determination, 50 μL was selected as the optimum volume and was applied in the subsequent experiments.

The vortex time (duration of the vortexing) is an important factor in VALLME that affects both the emulsification and the mass transfer processes, thus influencing the extraction efficiency. For the present study, the effect of the vortex time was studied over 1–5 min, the maximum speed setting of the vortex agitator (2800 rpm) was applied in all experiments. The results showed that the extraction efficiency increased markedly with the increase of vortex time from 1 min to 2 min. No significant effect was noted for vortex times ranging from 2 to 5 min, which indicated that the mass transfer of the analyte from the sample solution to the extraction solvent was so rapid that the extraction equilibrium was achieved in a short time, once sufficient extraction time has elapsed for equilibrium to be established, a further increase in extraction time does not affect the amount of analyte extracted. Therefore, 2 min was chosen as the extraction time for further experiments.

The effect of solution pH on the extraction was investigated in the pH range of 3–10 while holding the other variables constant. The results demonstrated that the EF increased upon increasing pH from 3 to 6, kept nearly constant within pH values of 6–8, but decreased markedly when at pH 10. So the sample solution did not require pH adjustment.

Analyte solubility in the aqueous phase typically decreases as the ionic strength increases, and the solubility of the extraction solvent in the sample decreases. Additions of varying concentrations of sodium chloride (NaCl, 0–8%, w/v) were evaluated to investigate the effect of the ionic strength on the extraction efficiency. No significant differences in the enrichment factors obtained as the ionic strength was modified. This may be explained that with the increase of the salt content, the viscosity and density of the solution increased, thus affected the mass transfer process negatively, and overcame the salting out effect [18]. This observation is in accordance with some previous reports of liquid–liquid microextraction [19]. Therefore, no salt was added in further experiments.

3.3. Analytical performance

The linearity, limit of detection (LOD), limits of quantification (LOQ) and repeatability of the proposed method were determined under the optimized conditions. A series of working solutions containing DMFu at five concentrations (0.01, 0.05, 0.1, 0.5, and 1.0 mg/kg) were prepared to establish a calibration curve. For each concentration, three extractions were performed. The results exhibit excellent linearity for analyte concentrations ranging from 0.01 to 1.0 mg/kg, the regression equation was $y=172857x-1002$ (correlation coefficients (R^2):0.998). The LOD and LOQ based on signal-to-noise ratios (S/N) of 3 and 10 were 0.0012 mg/kg and 0.0040 mg/kg, respectively. The LOQ of the proposed method is far below the MRLs established by the European Union, 2009/251/EC. The repeatability study was carried via six parallel replicate extractions with analysis at a concentration of 0.1 mg/kg. The intra-day and inter-day relative standard deviations (RSD) were 5.6% and 8.9%, respectively, demonstrating good repeatability in the proposed method. The recoveries obtained from two levels of DMFu spiking (0.1 and 0.5 mg/kg) in textile and leather samples ranged from 84 to 97% and 92 to 103%. The enrichment factor (EF) for the VALLME step was evaluated for three spiked solutions with varying DMFu concentration levels (0.005, 0.01, and 0.1 mg/L), the average EF being 53.

Table 1
Comparison of proposed ASE–VALLME–GC–MS with other methods for determination of DMFu.

Method	Sample	Organic solvent volume (mL)	Extraction time (min)	LOD (mg/kg)	ER (%)	Ref.
UASE[a]–SPE[b]–GC–MS	Consumer products	55	>30	0.0058	54	[1]
UASE–GC–MS	Desiccant and antimould sachets	0.5–2.0	5–10	0.005	89–109	[12]
UASE–HPLC	Desiccant and antimould sachets	>15	~15	0.024	98	[10]
UASE–GC–ECD[c]	Desiccant and mouldproof agents	0.5–2.0	5–10	0.014	91–98	[9]
UASE–GC–MS	Consumer products	10	~40	0.020	108–118	[11]
HS-SPME[d]–GC–MS	Consumer products	0	~45	0.010	82–89	[11]
ISO/TS 16186:2012	Footwear	10	60	0.100	/	[13]
ASE–VALLME–GC–MS	Textiles and leathers	0.06	25	0.0012	84–103	This method

[a] UASE=ultrasonic-assisted solvent extraction.
[b] SPE=solid phase extraction.
[c] ECD=electron-capture detection.
[d] HS-SPME=head space solid phase microextraction.

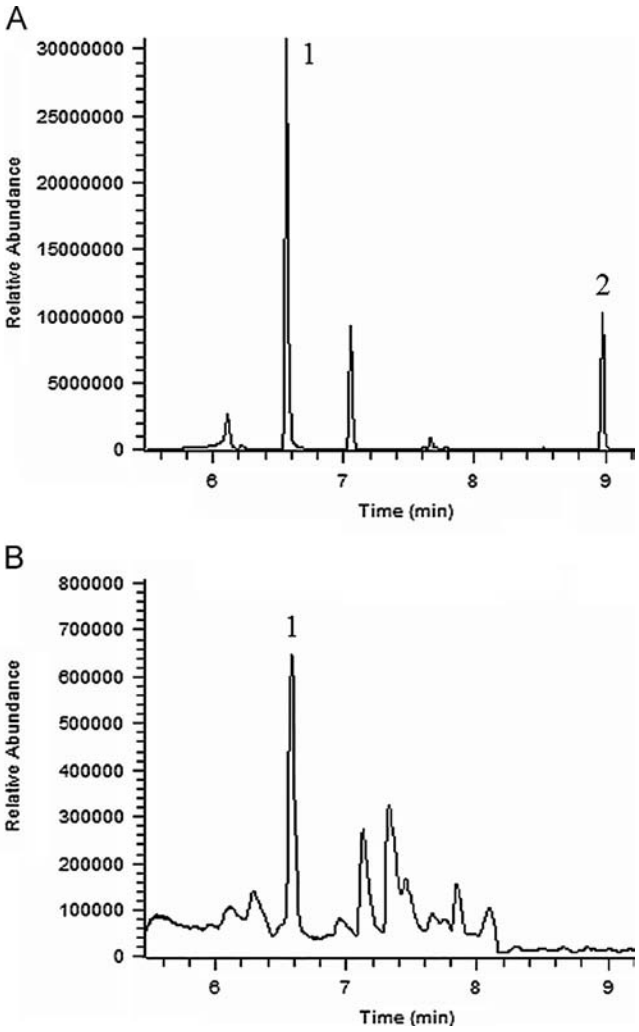

Fig. 1. Chromatograms of a positive leather sample (A) with proposed ASE–VALLME method and (B) with official method ISO/TS 16186:2012. (1) dimethyl fumarate and (2) naphthalene-d8.

The DMFu determined using our method was compared with that using other reported methods. As shown in Table 1, the proposed method has significant advantages over the other reported methods: (a) our method requires less extraction solvent and consequently produces less organic waste, making the procedure environmentally friendly; (b) we obtained a lower detection limit because VALLME includes a powerful preconcentration technique; and (c) the procedure is faster, as no time is needed for concentration with rotary evaporator [1,10,11] and no cleanup process with solid phase extraction [1,13].

3.4. Analysis of real samples

The method described herein was used to determine the DMFu in various textile and leather samples. Of the twenty samples, only three leather samples tested positive with concentrations ranging from 0.06 to 1.9 mg/kg. The results also indicate that the method is suitable for determining low DMFu quantities in textiles or leathers. As an example, Fig. 1 presents the chromatogram of a positive leather sample using the proposed method (A) and the official method ISO/TS 16186:2012 without a clean-up step (B). Comparison of the observed matrix effects shows that using water as an extraction solvent instead of organic solvents can relatively reduce the amounts of interfering contaminants (e.g. dyestuffs, auxiliaries, etc.) dissolved during the extraction period. The signal/noise ratio is lower in the proposed method due to higher capacity of cleanup and proconcentration.

4. Conclusions

In present work, two sample extraction techniques, ASE and VALLME, were combined to minimize the use of organic solvents, reduce the sample preparation time, and limit the manipulation of the samples, thus minimizing the risk of external contamination. The proposed method allows for the determination of low DMFu levels in textiles and leathers. The most important parameters involved in the ASE and VALLME were evaluated. The performance of the proposed method was investigated, and the sample matrix was found to have no adverse effects on the method's efficiency. The method demonstrates the advantages of simplicity, and minimized organic solvent consumption.

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