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Directly suspended droplet microextraction coupled with high performance liquid chromatography: A rapid and sensitive method for acetaldehyde assay in peritoneal dialysis fluids

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ABSTRACT

The aim of present study was to develop and validate a rapid, sensitive, inexpensive and reliable method for the detection of trace levels of acetaldehyde in peritoneal dialysis fluids (PDFs) by 2,4-dinitrophenylhydrazine (DNPH) derivatization and extraction. Separation and analysis of acetaldehyde via 2,4-dinitrophenylhydrazone (DNPH) was by reverse phase high performance liquid chromatography (HPLC). In order to remove co-eluting interferences and to pre-concentrate acetaldehyde, the extraction and clean-up of the sample has been performed using a liquid phase microextraction technique. In this research directly suspended droplet microextraction technique (DSDME) coupled with HPLC was used to determine acetaldehyde in PDFs. In DSDME method a free suspended droplet of an organic solvent (1-octanol) used as extraction phase. Important factors such as organic solvent, extraction time, droplet volume, sample and reagent solution volumes and rate of stirring were optimized. After extraction under optimal conditions the samples were analyzed by HPLC with UV detection at 360 nm. The linearity ranged from 0.01 to 100 mg L^{-1} with a relative standard deviation (RSD%; $n=3$) 5.6 Enrichment factor and limit of detection (LOD; $n=5$) were 54 and 1.12 μg L^{-1}, respectively.

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1. Introduction

During the standard heat sterilization process of PDFs, glucose degrades to form compounds called glucose degradation products such as acetaldehyde, formaldehyde, or glyoxal (see Fig. 1). This process occurred because carbohydrates are not stable when exposed to energy and they degrade into different molecules. The degree of degradation however depends on several factors other than the mode of sterilization, such as storage time, pH, light, glucose concentration, catalyst and temperature [1–5].

Aldehydes as a group are reactive compounds, capable of interacting with thiol and amino groups of proteins. Because of these properties Aldehydes may block SH-groups that are essential for cell division so act as cytotoxic agents [6,7].

There are evidences that these products may be responsible for some side effects such as impaired proliferation and impaired host defense mechanisms, demonstrated in vitro for a great variety of cells induced by commercially available PDFs [8–11]. These carbonyl compounds also promote irreversible advanced glycation end-products (AGEs), which might participate in the long-term remodeling of the peritoneal membrane [12].

Acetaldehyde was found to be the major reactive carbonyl compounds (RCCs) in PDFs. Acetaldehyde is present in the PDFs at low concentration levels. Because of its toxic effects on the biological systems even in trace amounts, its measurement in PDFs is essential. Therefore, a certain clean-up procedure for the complex sample matrix is necessary for the reliable and accurate analysis of acetaldehyde.

The most widely used derivatives for analysis of RCCs are 2,4-dinitrophenyl hydrazine (the corresponding hydrazones) derivatives because it forms stable hydrazone derivative for RCCs [13]. 2,4-Dinitrophenylhydrazine reacts with free carbonyls (Fig. 2) and can therefore serve as a marker for the extent of oxidative damage to a given protein [14].

Analysis of trace levels of RCCs such as acetaldehyde is very difficult because they are highly reactive, water soluble and volatile. Also they are major products of glucose degradation. Historically, liquid–liquid extraction (LLE) and solid-phase extraction (SPE) were often used for the extraction of hazardous compounds from aqueous matrices. However, these methods are time consuming, tedious, often require large amounts of organic solvent, and can be relatively expensive [15–17].

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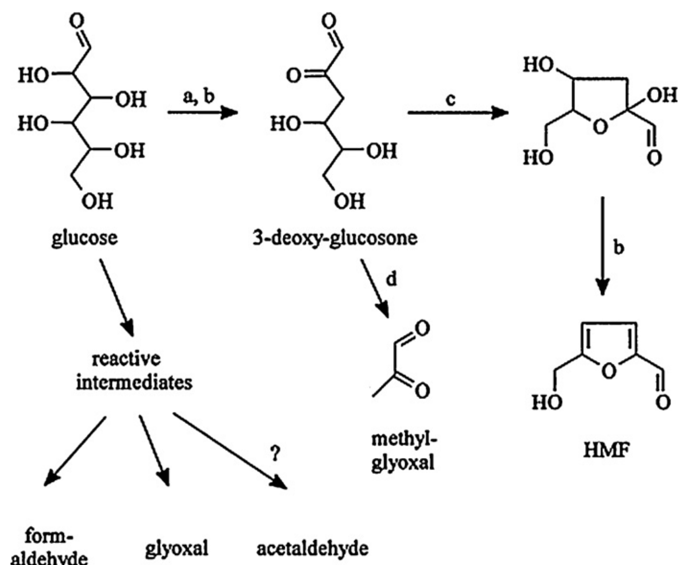

Fig. 1. Glucose degrades during the heat sterilization process of PDFs.

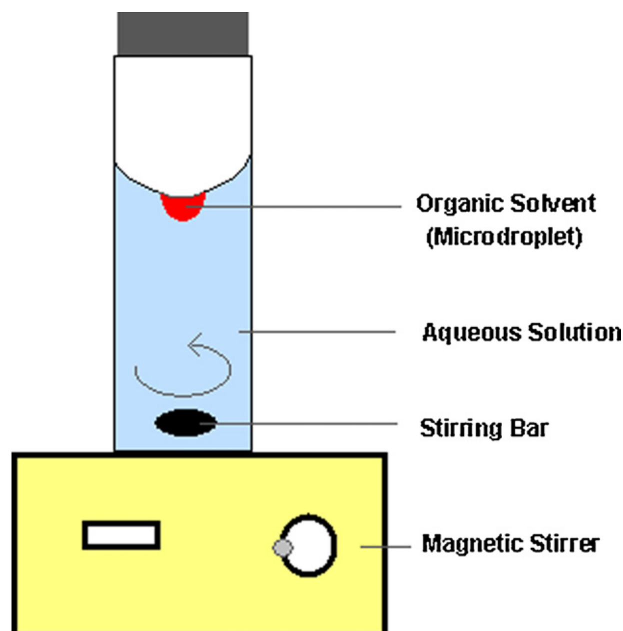

Fig. 3. DSDME device.

Therefore, the trend today, is toward the simplification and miniaturization of the sample preparation steps and a decrease in the quantities of organic solvents used [18,19]. Solid-phase microextraction (SPME) and liquid-phase microextraction (LPME) are of most recently developed sample preparation techniques.

Liquid-phase microextraction (LPME) has been recently introduced. It is a solvent-minimized sample pretreatment procedure of liquid–liquid extraction, in which only several micro liters of solvents are required to concentrate analytes from aqueous samples rather than several milliliters needed in LLE. This technique is not exhaustive and only a small fraction of analytes is pre-concentrated for analysis. Another important advantage is the integration of extraction and injection in one step, thus minimizing analysis time.

Apart from a wide choice of extraction solvents, LPME can be performed with the simplest devices, i.e. a traditional microsyringe and does not suffer from carry-over between extractions [20–23].

In this research we used a simple two phase LPME method under the name of directly suspended droplet microextraction (DSDME) [24–26]. In two-phase DSDME the donor phase is an aqueous solution providing feed. A stir bar is placed at the bottom of the aqueous sample rotating at a proper speed, which causes a weak gentle vortex or whirlpool in the solution. A small volume of an immiscible organic solvent is laid freely inside the organic phase by a microsyringe. The motion of the vortex results in the formation of a single microdroplet, near the center of rotation. The droplet itself may also rotate on the surface of the aqueous phase, increasing mass transfer [27] (see Fig. 3).

The analyte is extracted from the aqueous sample, into the organic acceptor droplet. After extraction, the droplet introduced into HPLC for further analysis.

Compared with the other LPME techniques based on droplet system, like single drop microextraction, DSDME offers several advantages over traditional extraction techniques for example more flexibility in the choice of the acceptor droplet volume and stirring frequency [28,29].

2. Experimental

2.1. Reagents and standards

1-Octanol and acetonitrile (HPLC grade) were obtained from Merck (Darmstadt, Germany). Analytical reagents grade 2,4-dinitrophenyl hydrazin were purchased from Merck too.

To prepare reagent solutions of DNPH, 12 g of DNPH was dissolved into 12 mL HCL (Conc.) and transferred it to a volumetric flask. 600 mL HCL (2 M) was added. It was then filtered and stored in a dark bottle.

The standard, was made of adding appropriate amount of acetaldehyde (about 10.0 mL) into 300 mL of above mentioned reagent solution, 2,4-dinitrophenylhydrazine (DNPH), drop wise until precipitation was completed. The acetaldehyde is converted to its hydrazone by reaction with 2,4-dinitrophenylhydrazine. The schismatic of reaction was depicted in Fig. 2. Copious crystalline orange yellow precipitates are formed. The precipitate was 24 h, dried in desiccators under vacuum. The precipitate after drying was re-crystallized from 95% ethanol.

A stock standard solution of 100 mg L^{-1} was prepared in methanol/water. Dilute it to give fresh working standard solutions in distilled water.

2.2. Instrumentation

The HPLC system used in this work was a Thermo Scientific Spectra SYSTEM liquid chromatography (San Jose, USA) and consisted of a Spectra System UV 2000 detector. A Perfectsil C18

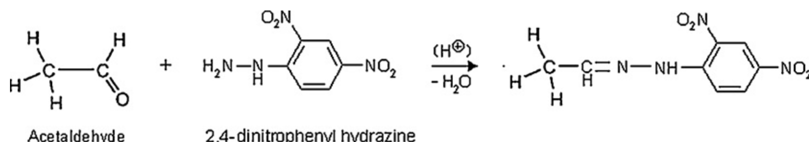

Fig. 2. Reaction between 2,4-dinitrophenylhydrazine (DNPH) and free carbonyls.

column (150 × 4.6 mm, 5 μm particle size) was used for separation. The degassed mobile phase consisting of acetonitrile–Water (49:51, v/v) was delivered by a Spectra System P4000 HPLC pump at 1.0 mL min^{-1}. The column was at ambient temperature (22 ± 0.5 °C).

In the extraction procedure, a 15-mL cylindrical sample vial, a 100-μL Hamilton HPLC syringe (Reno, NV) and a 10 mm × 2 mm stir bar were used.

2.3. Directly suspended droplet microextraction procedure

Acetaldehyde was determined as a hydrazone derivative. The samples were prepared by adding 0.5 mL aqueous solution of 2,4-DNPH to 7 mL of each sample. The reaction was allowed to develop for 15 min at room temperature.

The above solution was held in the 15-mL sample vial, and a stirring bar was adjusted within the sample solution. The magnetic stirrer was turned on and adjusted to a desired stirring speed. To make a steady and gentle vortex, it is important to keep the stirring bar rotates smoothly just at the center of the bottom. A microdroplet of an immiscible organic solvent (50 μL 1-octanol) was placed at the bottom of the vortex by microsyringe, and the syringe removed. After 4 min, organic droplet was withdrawn into the microsyringe and diluted with 200 μL acetonitrile and then 20 μL injected into the HPLC with UV detection at 360 nm for further analysis.

3. Results and discussion

Factors affecting the extraction efficiency such as organic solvent, the extraction time, microdroplet volume and stirring speed were optimized. The chromatography peak area which is related to the number of moles of analytes which is extracted into droplet was used to evaluate the extraction efficiency under different experimental conditions. Throughout these experiments the injection volume into HPLC is kept constant at 20 μL.

3.1. Choice of organic solvent

To establish a direct mode LPME technique, it is necessary to choose a proper organic solvent. The choice of the organic solvent needs following considerations; The solvent should have good affinity for target compounds, low solubility in water so as to prevent the dissolution in the aqueous phase and lower density than water. On the basis of these considerations 1-octanol, n-hexane and heptane were tested in the preliminary experiments. The peak area as extraction efficiency for each solvent is evaluated. The data indicates that 1-octanol gives the best extraction efficiency and was used as extraction solvent for the next extraction procedures.

3.2. Effect of extraction time

Like the other LPME procedures, DSDME is a technique dependent on equilibrium rather than exhaustive extraction. The amount of analyte extracted into the droplet at a given time depends upon the mass transfer of analyte from the aqueous phase into the organic solvent phase. This procedure requires a period of time for the equilibrium to be established. However it is not normally practical to use extraction times that are long enough for equilibrium to be established. Fig. 4 has shown the effect of extraction time on the method efficiency. For all analytes, on increasing the extraction time, the numbers of moles also increase. Since the extraction is not an exhaustive method a reasonable period of time (4 min) is selected for the subsequent experiments.

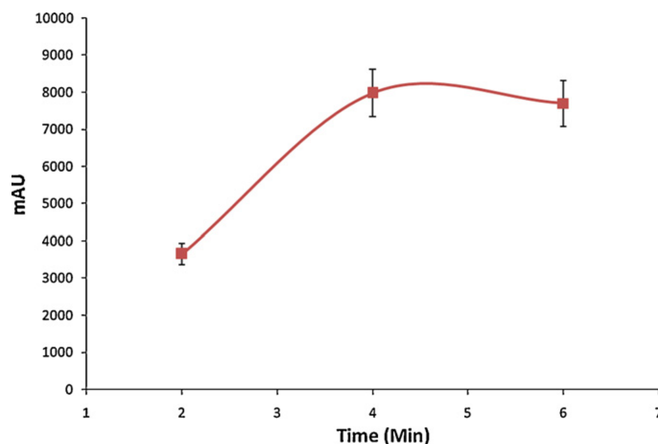

Fig. 4. Effect of extraction time on the DSDME.

3.3. Microdrop volume

The volume of extractor organic droplet has great effect on the extraction efficiency. The effects of 1-octanol drop size on the extraction were examined in the range of 30–70 μL. 70 μL droplet was unstable so the results were no repeatable. The relationship between volume of organic solvent and extraction efficiency was shown in Fig. 5. Thus 50 μL microdrop was chosen for further work. Optimization goal is the phase's ratio because the preconcentration factor (P.F.) was calculated based on the following equation [20–26]:

$$\text{P.F.} = \frac{A_{\text{RP, final}}}{A_{\text{PS, initial}}} \times \frac{V_d}{V_a}$$

where $A_{\text{RP, final}}$ and $A_{\text{PS, initial}}$ are the final and initial peak areas at after and before extraction of the analyte in organic solvent, respectively. V_d and V_a are volume aqueous sample and acceptor droplet.

3.4. Stirring speed

The agitation of the sample solution enhances the microextraction efficiency. In DSDME, the stirring speed has a direct influence on both the shape of the droplet and the mass transfer characteristics in the aqueous sample. The results were shown that the peak areas of all analytes increase with increasing stirring speed up to 650 rpm. It was also observed that the stirring speed above 800 rpm causes the instability, faster dissolution of the solvent droplet and decreases peak area. Hence, the stirring speed of 650 rpm was chosen as the optimum stirring rate.

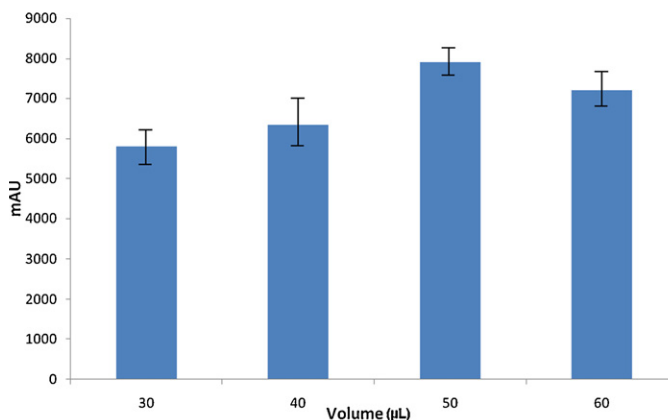

Fig. 5. Effect of microdroplet volume on the DSDME efficiency.

Table 1
DSDME performance and validation data.

Compound	Enrichment factor	R.S.D.% (n = 3)	Linear range ($\mu g L^{-1}$)	Correlation coefficient (R^2)	[a]LOD ($\mu g L^{-1}$) (n = 3)
Acetaldehyde	54	5.6	10–100,000	0.998	1.12

[a] L.O.D.s were examined at the S/N = 3.

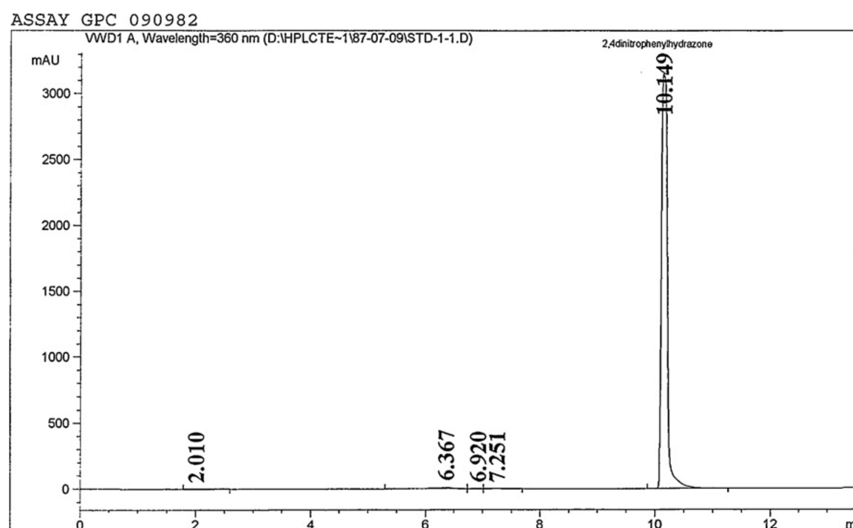

Fig. 6. Chromatogram of commercial PDFs contains acetaldehyde after DSDME extraction under optimal conditions. The separation conditions: feed volume; 7.5 mL, acceptor phase; 1-octanol, acceptor phase volume: 50 μL, extraction time; 4 min, stirring speed; 650 rpm.

Table 2
Acetaldehyde founded in commercial PD fluids.

Test solution	Acetaldehyde ($mg L^{-1}$)
PD fluid (after heat sterilized)	8.81–14.09
PD fluid (sterilized by filtration)	<0.88

4. Method validation

Under the optimized conditions, the analytical performance of the DSDME technique was evaluated. The calibration graph was plotted under the optimal extraction conditions as follows: sample solution: 7.5 mL, organic solvent: 1-octanol, extraction time: 4 min, stirring speed: 650 rpm, microdroplet volume: 50 μL.

The precision of the method was determined using replicate analysis (n = 3) of analyte in five different sources of peritoneal dialysis fluids at all concentrations utilized for the construction of calibration curve. Repeatability (R.S.D.%, n = 3) evaluated with three replicated experiments.

Table 1 shows relative standard deviations, dynamic linear ranges (DLR), limits of detection (LOD) and correlation coefficients (R^2) obtained by the method. Limit of detection was calculated experimentally as the minimum analyte concentration providing chromatography signals three times higher than background noise.

Practical pre-concentration factors were calculated as the proportion peak areas after extraction to before that.

5. Real samples analysis

The DSDME technique was applied for determination of acetaldehyde in PDFs. These samples were produced in the Daroo Sazi Samen Co., Mashhad, Iran. Acetaldehyde was detected in PDFs samples. This method was perfectly selective for acetaldehyde in PDFs (see Fig. 6 and Table 2).

LPME is not exhaustive extraction method, so the relative recovery defined as the ratio of the concentrations found in the investigated matrix (PDFs) to those in deionized water spiked with the same amounts of analytes. It is generally used instead of absolute recovery. The recovery values close to 100% indicate the lack of matrix effect and good accuracy of the procedure.

The relative recovery of acetaldehyde in PD fluid (sterilized by filtration) was determined as the ratio of the concentration in the real sample and deionized water samples spiked at the same concentration level (1.0 $mg L^{-1}$).

Relative recovery percent of 82.8 ± 4.9 was calculated for acetaldehyde in the PD Fluids.

Relative recovery experiments were performed on the same PD fluids which were sterilized by filtration because as was mentioned

Table 3
Comparison of some methods which were used for determination of acetaldehyde.

Matrix	Method	RSD (%)	LOD	Linearity	Ref.
Blood	GLC		0.1 ($\mu g mL^{-1}$)		[15]
Water	GC	5.8 (n = 6)	0.02 ($mg L^{-1}$)		[30]
Yogurt	GC (Head space)	–	31.1 ($\mu g g^{-1}$)	79–790 (μg)	[31]
Pet bottles	GC	–	0.2 ($\mu g L^{-1}$)	4.35–43.5 ($\mu g L^{-1}$)	[32]
Fuel	HPLC	5.6 (n = 5)	2.03 ($\mu g L^{-1}$)	3–300 ($mg L^{-1}$)	[33]
Liquors	IAD[a]	2.6 (n = 7)	0.1 (μM)	0.2–10 (μM)	[34]

[a] Immobilized aldehyde dehydrogenize.

in Fig. 6 and Table 2, heat sterilized PDFs was containing relatively large amount of acetaldehyde.

6. Conclusion

The directly suspended droplet microextraction (DSDME) method developed in this study was found to be a suitable method for routine control of acetaldehyde in peritoneal dialysis fluids (PDFs). On the other hand, since acetaldehyde was not stable in the water matrix it measurement in terms of 2,4-dinitrophenylhydrazone (DNPH) resulting in an accurate measurements of acetaldehyde in PDFs.

Our results also indicate that the major reason for the degradation of PDFs into these compounds is the heat-sterilization procedure.

The results obtained with the method described above indicate that DSDME method is a good alternative extraction technique for determination and detection of trace amounts of acetaldehyde in PDFs and offers highly interesting advantage from an analytical point of view such as rapidness, sensitivity, inexpensively, reliability and no pollution with low limit of detection.

In this method, contrary to the conventional single drop liquid-phase microextraction technique, a droplet is directly suspended on the surface of the donor phase, without using a microsyringe as supporting device. Therefore, a larger droplet with a higher lifetime than conventional one can be used. Thus, as the drop surface increases with the increase of the drop volume, this results in a larger enrichment factor. On the other hand, this large and self-stable droplet is freely suspended in the feed solution and can be rotated around a symmetrical axis during the extraction procedure, which causes an increase in mass transfer process and decrease in equilibrium time. Compared to the most conventional extraction procedures, this extraction technique is very fast, easy and simple.

This technique was successfully used for the separation and preconcentration of acetaldehyde in PDFs. The method was compared with the other previous works (Table 3).

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