# Gas Chromatography-flame Ionization Determination of Benzaldehyde in Non-Steroidal Anti-inflammatory Drug Injectable Formulations using new Ultrasound-assisted Dispersive Liquid-liquid Microextraction

[1]HOSSEIN ALI MASHAYEKHI*, [2]MOHAMMAD REZAEE, [3]SHIRIN SADEGHI GARMAROUDI, [1]KHALIL POURSHAMSIAN AND [2]SEYED JAVAD AHMADI

[1]*Department of Chemistry, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran.*

[2]*Nuclear Fuel Cycle Research School, Nuclear Science and Technology Research Institute, Atomic Energy Organization of Iran, P.O. Box 14395-836, Tehran, Iran.*

[3]*Department of Chemistry, Science and Research Branch, Islamic Azad University, Tehran, Iran.*

chem.mashayekhi@tonekaboniau.ac.ir*

(Received on 28th June 2011, accepted in revised form 30th November 2011)

**Summary**: In this study, simple and efficient ultrasound-assisted dispersive liquid-liquid microextraction combined with gas chromatography (GC) was developed for the preconcentration and determination of benzaldehyde in injectable formulations of the non-steroidal anti-inflammatory drugs, diclofenac, Vitamin B-complex and Voltaren injection solutions. Fourteen microliters of toluene was injected slowly into 10 mL home-designed centrifuge glass vial containing an aqueous sample without salt addition that was located inside the ultrasonic water bath. The formed emulsion was centrifuged and 2 µL of separated toluene was injected into a gas chromatographic system equipped with a flame ionization detector (GC-FID) for analysis. Several factors influencing the extraction efficiency as the nature and volume of organic solvent, extraction temperature, ionic strength and centrifugation time were investigated and optimized. Using optimum extraction conditions a detection limit of 0.3 µg $L^{-1}$ and a good linearity in a calibration range of 2.0-1000 µg $L^{-1}$ were achieved for analyte. This proposed method was successfully applied to the analysis of benzaldehyde in three injection formulations and relative standard deviation (RSD) of analysis (n=3), before spiking with standard benzaldehyde were 3.3, 2.0 and 1.3% for Na-diclofenac, vitamin B-complex and voltaren, respectively and after spiking of standard benzaldehyde (0.3 mg $L^{-1}$), the RSD were 6.5, 3.6 and 2.8% for Na-diclofenac, vitamin B-complex and voltaren, respectively.

## Introduction

Presence of potentially toxic quantities of benzaldehyde in some generic injection formulations of Na-diclofenac was reported [1]. This arises from oxidation of benzyl alcohol, which is used in concentrations up to 2% as an antimicrobial preservative. Benzyl alcohol at concentration of 0.9-2.0% is commonly used as an antibacterial agent in many pharmaceutical formulations especially intended for intravenous administration [2]. Benzyl alcohol is widely used in organic synthesis and as a solvent for various compounds, for example cellulose [3]. Intraventricular hemorrhage and death in preterm neonates has been associated with the use of fluid containing benzyl alcohol. Exposure to benzyl alcohol was significantly associated with the development of kernicterus [2]. Benzyl alcohol preservatives in intravascular flash solutions has been reported to cause neurological deterioration and death in low birth weight infants [4]. In a single year (2000) nearly 200 cases of transient or permanent paraplegia had resulted following intramuscular injection of generic brands of Na-diclofenac, which contained benzyl alcohol as preservative. Most commonly, the paralysis developed rapidly, often with pain and anaesthesia, which occurred immediately or with a delay after the intramuscular injection, though the causative agent has not been positively identified [5-7]. Benzyl alcohol intended for use in the manufacture of parenteral dosage forms should not contain more than 0.05% of benzaldehyde quantifiable by gas chromatography [8]. The United States Pharmacopoeia does not contain a monograph on injectable benzyl alcohol solutions, but limits the presence of benzaldehyde in benzyl alcohol to levels of 0.2%, with quantification by HPLC [9].

Dispersive liquid-liquid microextraction (DLLME) was introduced by Rezaee *et al.* in 2006 [10]. In this technique very fine droplets are produced by dispersion of an appropriate mixture of extraction and disperser solvents in an aqueous sample. The contact surface between phases is markedly increased, which reducing the extraction time and increasing the preconcentration factor. However, consumption of disperser solvent in DLLME have lead to some disadvantages such as decreasing of partition coefficient of analyte into extracting solvent, increasing cost as well as environmental pollution and limits variety of solvents [11-17].

*To whom all correspondence should be addressed.

Saleh *et al.* applied low-density organic solvent by using special vessel for the determination of PAHs in water samples [18]. Rezaee *et al.* used low-density extraction solvent by using special vessel for the determination of methyl-tert-butyl ether (MTBE) [19]. In the present study, a simple and fast new ultrasound-assisted dispersive liquid-liquid microextraction method based on dispersion of micro volumes of organic solvents (e.g. toluene, 1-octanol, undecanol and dodecanol) in injectable formulation samples for the determination of benzaldehyde was developed. Home-designed centrifuge glass vials containing an aqueous sample were immersed into an ultrasonic water bath. The micro volumes of organic solvents were withdrawn into a microsyringe and injected slowly into the sample through the capillary tube at the top of the centrifuge vial. After dispersion, two phases can be readily separated by centrifugation. The conic top of centrifuge vial attached to a capillary tube makes it suitable for easy collection of micro volumes of the floated organic solvent on the surface of the aqueous sample.

**Results and Discussion**

In the present study, a new ultrasound-assisted dispersive liquid-liquid microextraction was investigated for preconcentration and determination of benzaldehyde from injection formulations. The influences of various parameters such as the kind and volume of the extraction solvent, ionic strength, extraction temperature and centrifugation time on the extraction efficiency were studied. A univariate approach was employed to optimize the influential factors in this method.

*Solvent Extraction*

The selection of an extracting solvent is of great importance in solvent microextraction methods in order to obtain efficient extraction. Some factors should be considered, namely they must have low water solubility, be able to extract the analytes of interest and compatible with the analytical instrumentation to be used. The amount of solvents 20.0, 14.0, 12.0 and 10.0 µL of 1-octanol, toluene, 1-dodecanol and 1-undecanol respectively were optimized and dispersed into 10 mL of aqueous sample containing 100 µg $L^{-1}$ of benzaldehyde. A part of two microliters of each collected extraction solvent was injected into the GC-FID system for subsequent analysis. The results were shown in the Fig. 1 and indicated that toluene has the highest extraction efficiency for determination of benzaldehyde.

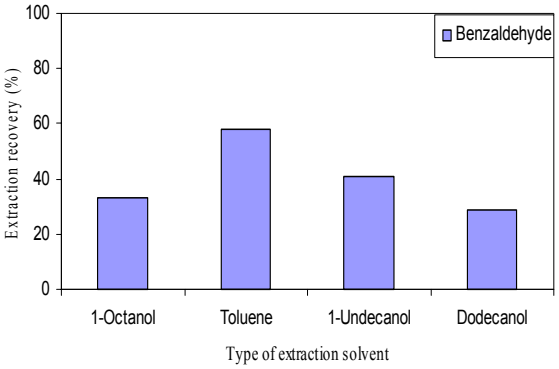


Fig. 1: Effect of type of extraction solvent on the extraction recovery of analyte. Extraction conditions: water sample volume, 10.0 mL; extraction solvent volumes, 14.0 µL toluene, 20.0 µL 1-octanol, 12.0 µL dodecanol, 10.0 µL 1-undecanol; concentration of analyte, 100 µg $L^{-1}$.

*Influence of Centrifugation Time*

Centrifugation was required to break down the emulsion and accelerate the phase-separation process. Centrifugation times were at 3500 rpm and examined in the range of 0-20 min. The volumes of collected toluene from the surface of aqueous samples were measured and used as the response factor. Results showed that the volume of solvent was maximum obtained as the centrifugation time increased from 0 to 10 min. The evaporation of toluene observed at longer centrifugation times (>15 min). Accordingly, 10 min selected as the optimum value.

*Influence of Ionic Strength*

The salting out effect has been universally used in SPME and LLE methods. The addition of salt to an analytical sample can potentially increase the analyte extraction recovery in the microextraction procedures. The effect of the ionic strength on the extraction efficiency was evaluated by increasing NaCl concentrations in the range of 0-8 % (w/v) in water samples containing 100 µg $L^{-1}$ of benzaldehyde. In the increasing concentration of NaCl, extraction efficiency of benzaldehyde did not changed significantly; this is possibly because of two opposite effects of addition of salt in DLLME method. One is to increase the volume of collected solvent, because of the decrease of solubility of the extraction solvent in the presence of salt and also, salt causes decreasing the dispersion efficiency, which reduce the extraction efficiency; another effect is the

salting-out effect, which increases the extraction efficiency (Fig. 2). Therefore, further extractions were performed without salt.

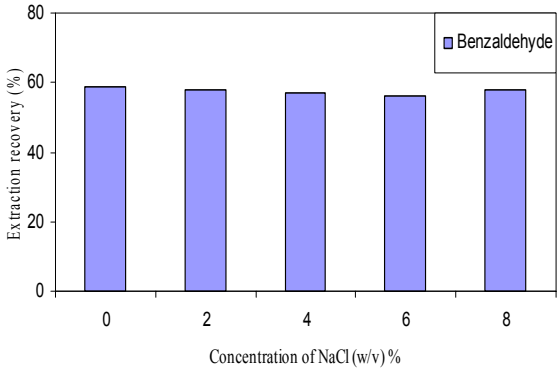


Fig. 2: Effect of salt addition on the extraction efficiency. Conditions: sample solution: 10 mL of 100 µg $L^{-1}$ of analyte in doubly distilled water; volume of organic solvent: 14.0 µL; solution temperature: 25 ± 3 ºC; dispersion time: 30 second; centrifugation time: 10 min.

*Influence of the Volume of the Extracting Solvent*

The effect of volume of extracting solvent in the proposed method of benzaldehyde was also investigated at five levels in the range of 12-50 µL. Volumes smaller than 10 µL were dissolved in aqueous bulk. The minimum collectable volume of organic solvent in the designed system was 2 µL (12 µL of emulsified toluene). As shown in Fig. 3, the concentration of benzaldehyde in the organic phase decreased by increasing of the volume of the organic phase due to the dilution effect. Results showed that maximum preconcentration was achieved by using 12 µL of toluene. But, due to the difficulty in collection of 2 µL of the floated toluene that produced poorer precision, the volume of 14 µL was chosen as the optimum volume of the organic solvent.

*Influence of Extraction Temperature*

Emulsification phenomenon, distribution coefficient and mass transfer of target analyte can be affected by temperature. To determine the effect of sample solution temperature during emulsification-extraction process, 10 mL aqueous solution containing 100 µg $L^{-1}$ of benzaldehyde was extracted with 14.0 µL toluene at different temperatures ranging from 25 to 50 ºC. The temperature has no significant effect on the extraction efficiency of the benzaldehyde, because of the contact surface between organic solvent and the sample is very large and there is no limiting effect caused by slow mass transfer. Therefore, it is clear that emulsification temperature do not affect the extraction efficiency of benzaldehyde. Accordingly, in further experiments emulsification-extractions were conducted at room temperature (25 ± 3 °C).

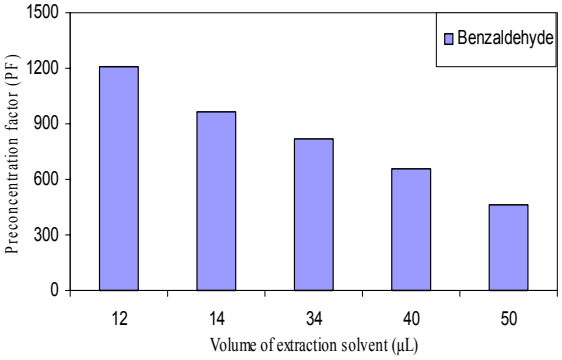


Fig. 3: Effect of extracting solvent volume on the extraction efficiency. Conditions: sample solution: 10 mL of 100 µg $L^{-1}$ of analyte in doubly distilled water without salt; solution temperature: 25 ± 3 ºC; dispersion time: 30 second; centrifugation time: 10 min.

*Influence of Extraction Time*

The extraction time is an interval time started after dispersion and ended just before centrifugation. The effect of time on the extraction efficiency was examined in the range of 0-40 min. Extraction time has no significant effect on the extraction efficiency of benzaldehyde, because of the contact surface between extracting solvent and sample was infinitely larger and equilibrium state was achieved during a few second. The comparison of equilibrium time of the proposed method and some other reported microextraction methods [20, 21] for extraction of benzaldehyde indicates that this novel method has a very short equilibrium time comparing to the other extraction methods. Therefore, in further experiments the centrifugation was carried out just after dispersion process.

*Analytical Performance*

The merit of developed method under optimized conditions is shown in Table-1. Linearity was observed over the range 2.0-1000 µg $L^{-1}$ and Coefficient of determination ($r^2$) 0.9989. The extraction recovery was 58.0 % and preconcentration factor was 966 in this method for benzaldehyde. The

relative standard deviation (RSD, n = 4) at the level of 20.0 µg $L^{-1}$ of benzaldehyde was 6.7%. The limit of detection (LOD), based on signal to noise ratio (*S/N*) of 3 were 0.3 µg $L^{-1}$.

Table-1: Quantitative results of ultrasound-assisted DLLME and GC-FID method for benzaldehyde.

| $r^{2e}$ | ER (%)[d] | PF[c] | RSD (%)[b] | LOD[a] (µg/L) | Linear range (µg/L) | Analyte |
|---|---|---|---|---|---|---|
| 0.9989 | 58.0 | 966 | 6.7 | 0.3 | 2.0-1000 | Benzaldehyde |

[a]LOD, limit of detection for S/N=3.
[b]RSD, relative standard deviation (n = 4).
[c]Preconcentration factor at the concentration analyte of 100 µg $L^{-1}$.
[d]Extraction Recovery
[e]coefficient of determination

*Analysis of Real Samples*

To test the applicability of the proposed method, three injection formulations were extracted and analyzed. In order to reduce the matrix effect; three real samples were diluted with deionized water to 1:10, spiked with benzaldehyde standard (0.3 mg $L^{-1}$ concentration level) to assess matrix effect. The recoveries were between 90.0 and 96.6% (Table-2) and show that matrix has negligible effect. Fig. 4-6 show GC-FID chromatograms, prior (a) and after (b) spiking with benzaldehyde at 0.3 mg $L^{-1}$ level in Voltaren, Vitamin B-Complex and Na-diclofenac samples, respectively.

Table-2: Determination of benzaldehyde (BZH) in three injection formulation solutions samples.

| Retention Time of BZH (min) | Relative Recovery (%) | Found BZH ( mg $L^{-1}$) ± RSD , n=3 | Added BZH (mg $L^{-1}$) | Concentration of BZH (mg $L^{-1}$) ± RSD n=3 | Sample |
|---|---|---|---|---|---|
| 5.1 | 90.0 | 0.78 ± 6.5 | 0.30 | 0.51 ± 3.3 | Na-diclofenac |
| 5.1 | 93.3 | 0.51 ± 3.6 | 0.30 | 0.23 ± 2.0 | Vitamin B-complex |
| 5.1 | 96.6 | 0.44 ± 2.8 | 0.30 | 0.15 ± 1.3 | Voltaren |

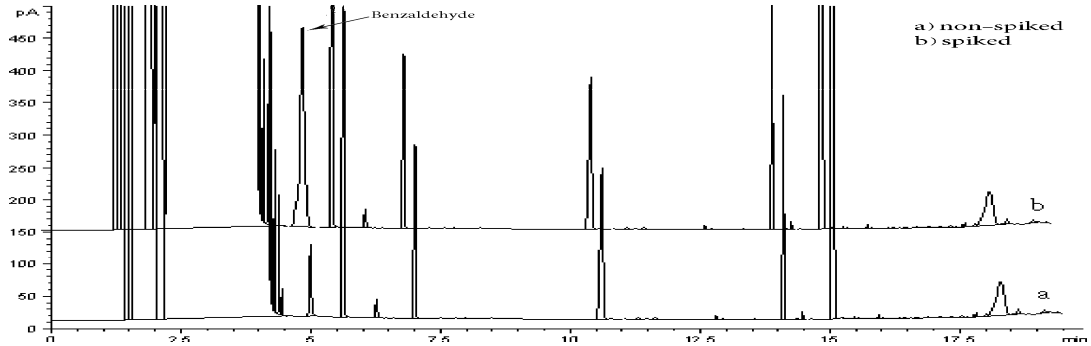


Fig. 4: GC-FID chromatograms of analyte in Voltaren (a) before spiking and (b) after spiking with 0.3 mg $L^{-1}$ benzaldehyde using proposed method combined with GC-FID under optimum conditions.

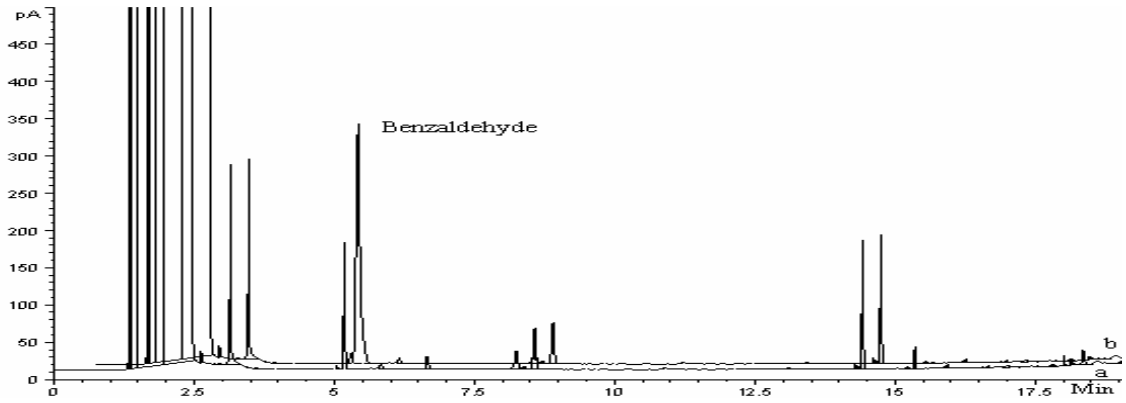


Fig. 5: GC-FID chromatograms of analyte in Vitamin B-Complex (a) before spiking and (b) after spiking with 0.3 mg $L^{-1}$ benzaldehyde using proposed method combined with GC-FID under optimum conditions.

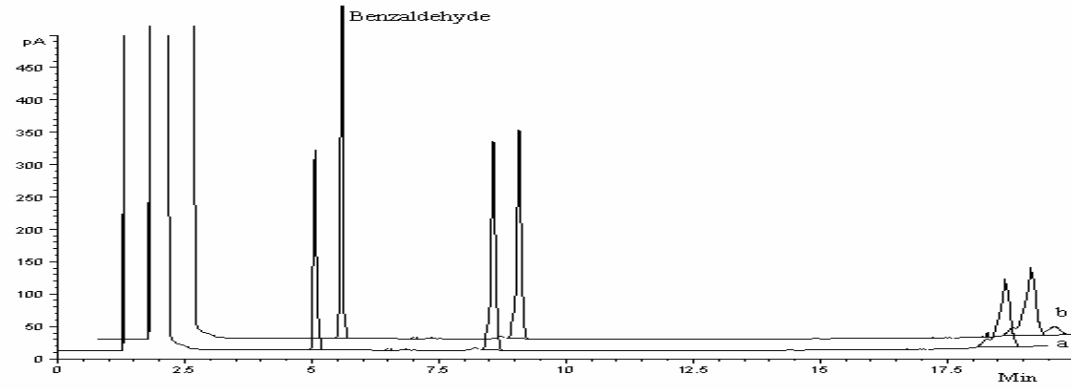


Fig. 6: GC-FID chromatograms of analyte in Na-diclofenac (a) before spiking and (b) after spiking with 0.3 mg $L^{-1}$ benzaldehyde using proposed method combined with GC-FID under optimum conditions.

## Experimental

*Chemicals and Reagents*

All chemicals were of analytical reagent grade. Benzaldehyde, toluene, 1-octanol, 1-undecanol, 1-dodecanol were obtained from Merck (Darmstadt, Germany). Reagent grade NaCl was also obtained from Merck. Double distilled water was used for preparation of aqueous solutions. Various batches of generic Na-diclofenac, Vitamin B-complex and Voltaren injection formulations solutions were kindly supplied by manufacturers in Iran: EXIR-IRAN, DAROU PAKHSH.

*Apparatus*

A 40 kHz and 0.138 kW ultrasonic water bath with temperature control (Tecno-Gaz SpA, Italy) was applied to emulsify the organic solvent. Five hundred and twenty five µL Hamilton syringes (Bonaduz, Switzerland) were used to inject the organic solvent into aqueous samples. Twenty milliliters home-designed centrifuge glass vials were used for extraction and collection procedure (Fig. 7). A 10.0 µL Hamilton gas-tight syringe was applied for collection of floated organic solvent and injection into the GC. A gas chromatograph (Agilent GC-7890) equipped with a split/splitless injector system and flame ionization detector, was used for separation and determination of benzaldehyde. Ultra pure helium gas (99.999%, Air products, UK) was passed through a molecular sieve and oxygen trap (Crs, USA) was used as carrier gas with flow rate of 2 mL $min^{-1}$. The injection port was held at 250 °C, operated in the splitless mode for 1 min and then split valve was opened– for split ratio of 1:5. Separation was carried out on a DB5, 25 m × 0.32 mm i.d. and 0.25 µm film thickness from SGE (Victoria, Australia) Capillary column. The oven temperature was kept at 70 °C for 3 min and then increased to 100 ºC at the rate of 5 ºC/min, and was held for 2 min and then increased to 250 °C at the rate of 20 °C/min and was held for 1 min. The FID oven temperature was maintained at 270 ºC. Hydrogen was generated by hydrogen generator (OPGU-2200S, Shimadzu) for FID at a flow rate of 30 mL $min^{-1}$. The flow of air (99.999%, Air products) for FID was 400 mL $min^{-1}$. The model 2010 D centurion scientific centrifuge (Westsussex, UK) was used for separation of floated phase from sample solution.

*Ultrasound-assisted DLLME Procedure*

Fig. 7 shows the schematic procedure of the proposed method. The home-designed centrifuge glass vial was filled by the aqueous sample up to the middle of the conic head of the vial (Fig. 7, a). Appropriate volume of 100.0 mg $L^{-1}$ of stock solution of benzaldehyde was added to the aqueous sample without salt addition. The vial was immersed into an ultrasonic water bath. 14.0 µL of organic solvent (toluene) was slowly injected into the water sample by a 25 µL syringe (Fig. 7, b) during switch on ultrasonics. After a thirty second sonication at 40 kHz of ultrasound frequency and 0.138 kW of power at 25 ± 3 °C, the formed emulsion was centrifuged at 3500 rpm for 5 min to separate the phases. After separation of the two phases, a few microliters of doubly distilled water were added into the vial through the glass tube fixed on the side of the vial (Fig. 7, c). The floated organic solvent was raised into the capillary tube attached to the top of the vial and collected by a gas-tight syringe (Fig. 7, d). Two microliters of organic solvent was injected into GC-FID instrument.

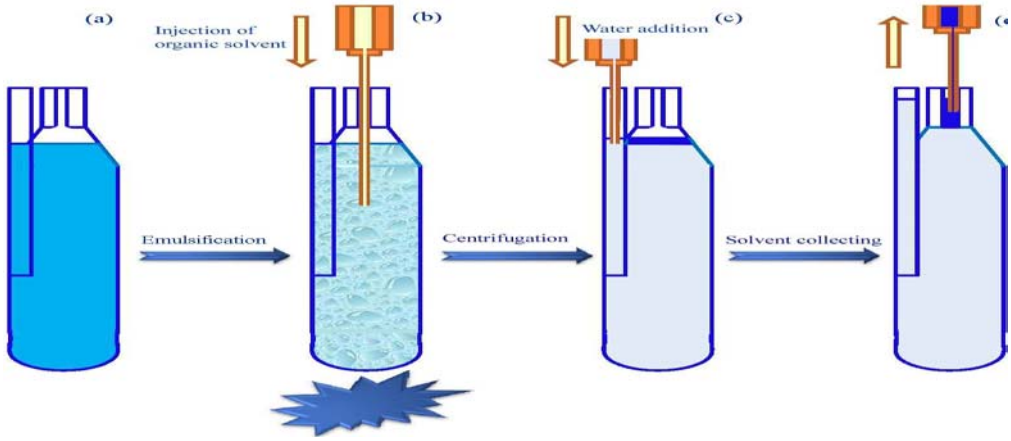


Fig. 7: Schematic representation of the proposed method (a) Aqueous sample solution in the home-designed emulsification glass vial without salt addition, (b) simultaneous injection and dispersion of 14.0 µL toluene into aqueous sample, (c) addition of a few µL of doubly distilled water into the vial and (d) collection of toluene transferred into the capillary tube at the top of the vial (about 6 µL).

## Conclusions

A simple and reliable new ultrasound-assisted dispersive liquid-liquid microextraction method was developed for the rapid concentration and determination of benzaldehyde in injectable formulations of the non-steroidal anti-inflammatory drugs, diclofenac, Vitamin B-complex and Voltaren solutions. An ultrasound-assisted process was applied to accelerate the formation the cloudy solution, which was markedly increased the extraction efficiency and reduced the equilibrium time. The developed method was sensitive, reproducible and linear over a wide range. The performance of this procedure in benzaldehyde extraction from three injection formulations solutions was excellent and no matrix effect was observed.

## Acknowledgement

Financial support by Tonekabon Branch, Islamic Azad University (Tonekabon, Iran) for the support during the period of this research is gratefully acknowledged.

## References

1. A. G. Kazamifard, D. E. Moore and A. Mohammadi, *Journal of Pharmaceutical and Biomedical Analalysis*, **30**, 257 (2002).
2. D. S. Jardine and J. Rogers, *Pediatrics*, **83**, 153 (1989).
3. R. C. Baselt and R. H. Cravey (Editors), *Dispersion of Toxic Drugs and Chemical in Man*, *Chemical Toxicology Institute*, Foster City, CA, 4th ed., 78 (1995).
4. J. L. Hiller, G. I. Benda, M. Rahatzad, J. R. Allen, D. H. Culver, C. V. Carlson and J. W. Reynolds, *Pediatrics*, **77**, 500 (1986).
5. J. H. Saiki, S. Thompson, F. Smith and R. Atkinson, *Cancer*, **29**, 370 (1972).
6. A. F. Hahn, T. E. Feasby and J. J. Gilbert, *Neurology*, **33**, 1032 (1983).
7. D. Duncan and W. H. Jarvis, *Anesthesiology*, **4**, 465 (1943).
8. The British Pharmacopoeia, HMSO, London, 161 (1998).
9. The United States Pharmacopoeia 24 and the National Formulary 19, USP Commission, Rockville, MD, USA, 2420 (2000).
10. M. Rezaee, Y. Assadi, M. R. Milani Hosseini, E. Aghaee, F. Ahmadi and S. Berijani, *Journal of Chromatography* A, **1116**, 1 (2006).
11. R. R. Kozani, Y. Assadi, F. Shemirani, M. R. Milani Hosseini and M. R. Jamali, *Talanta*, **72**, 387 (2007).
12. M. A. Farajzadeh, M. Bahram and J. A. Jonsson, *Analytica Chimica Acta*, **591**, 69 (2007).
13. A. Bidari, E. Zeini Jahromi, Y. Assadi and M. R. Milani Hosseini, *Microchemical Journal*, **87**, 6 (2007).
14. N. Shokoufi, F. Shemirani and Y. Assadi, *Analytica Chimica Acta*, **597**, 349 (2007).
15. M. Rezaee, Y. Yamini, S. Shariati, A. Esrafili and M. Shamsipur, *Journal of Chromatography* A, **1216**, 1511 (2009).
16. M. Rezaee, Y. Yamini and M. Faraji, *Journal of Chromatography* A, **1217**, 2342 (2010).

17. Y.Yamini, M. Rezaee, A. Khanchi, M. Faraji and A. Saleh, *Journal of Chromatography* A, **1217**, 2358 (2010).
18. A. Saleh, Y. Yamini, M. Faraji, M. Rezaee and M. Ghambarian, *Journal of Chromatography* A, **1216**, 6673 (2009).
19. M. Rezaee, Y. Yamini, H.A. Mashayekhi, M.H. Naeeni and M. Bashiri Jubari, *Journal of the Chinese Chemical Society*, **58**, 332 (2011).
20. A. Dasgupta and P. E. Humphrey, *Journal of Chromatography* B, **708**, 299 (1998).
21. A. G. Kazamifard, D. E. Moore, A. Mohammadi and A. Kebriyaeezadeh, *Journal of Pharmaceutical and Biomedical Analysis*, **31**, 685 (2003).