Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis
31 (2003) 685–691
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Capillary gas chromatography determination of benzaldehyde arising from benzyl alcohol used as preservative in injectable formulations

Amir G. Kazemifard [a], Douglas E. Moore [b,*], A. Mohammadi [a], A. Kebriyaeezadeh [a]

[a] College of Pharmacy, Medical Sciences University of Tehran, Tehran, Iran
[b] Faculty of Pharmacy, University of Sydney, Sydney, NSW 2006, Australia

Received 17 June 2002; accepted 21 October 2002

Abstract

A simple, precise and accurate capillary gas–liquid chromatographic procedure has been developed to determine benzaldehyde, the toxic oxidation product of the widely used preservative and co-solvent benzyl alcohol, in injectable formulations of the non-steroidal anti-inflammatory drugs, diclofenac and piroxicam, as well as in Vitamin B-complex injection solutions. Following liquid–liquid extraction with chloroform, separation and quantification are achieved on a fused silica capillary column (25 m × 0.53 mm i.d.) coated with 0.5 μm film of OV-101. 3-Chlorobenzaldehyde was used as internal standard with flame-ionization as the detection mode. The ability of the system to resolve benzaldehyde peak from interfering components is good. The method displays excellent linearity over the concentration range 0.5–100 μg/ml of benzaldehyde and a precision of better than 2.5% from intra- and inter-day analyses. The quantification limit for benzaldehyde is 0.4 μg/ml. Levels of benzaldehyde in generic diclofenac and piroxicam injection formulations were found to be seven to 15 times higher than in reference formulations, and double in generic Vitamin B-complex injection formulations.

Keywords: Benzaldehyde; Benzyl alcohol; Injectable formulations; Na-diclofenac; Piroxicam; Vitamin B-complex; Capillary gas–liquid chromatography

1. Introduction

Recently we reported on the presence of potentially toxic quantities of benzaldehyde in some generic injection formulations of Na-diclofenac [1]. This arises from oxidation of benzyl alcohol, which is used in concentrations up to 2% as an antimicrobial preservative. We developed a rapid

* Corresponding author. Tel.: +61-2-9351-2334; fax: +61-2-9351-4391.
E-mail address: demoore@pharm.usyd.edu.au (D.E. Moore).

0731-7085/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0731-7085(02)00729-X

and sensitive polarographic method capable of quantifying benzaldhyde to 50 ng/ml in Na-diclofenac injection samples. This was done in response to a warning, issued to hospitals and medical practitioners by the Iranian Ministry of Health. In a single year (2000) nearly 200 cases of transient or permanent paraplegia had resulted following intramuscular injection of generic brands of Na-diclofenac (and in a few cases piroxicam), both of 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 [2–4].

In the polarographic analysis there was no interference from diclofenac, but it was found that the method is not extendable to other injection formulations containing piroxicam or Vitamin B-complexes due to overlap of reduction peaks arising from the drugs. As a consequence it became necessary to examine the chromatographic methods as an alternative approach to determining benzaldehyde in the presence of the other anti-inflammatory drugs or vitamins.

The United States Pharmacopoeia limits the presence of benzaldehyde in benzyl alcohol to levels of 0.2%, with quantification by HPLC [5]. This method has not proven to be extendable to benzyl alcohol containing injections because of active drug interferences. The British Pharmacopoeia states that benzyl alcohol intended for use in the manufacture of parenteral dosage forms should not contain more than 0.05% of benzaldehyde and describes a GC method for its determination in the raw material [6]. Other GC methods for the determination of traces of benzaldehyde in benzyl alcohol have been reported [7–10], but none of these have been validated for application to pharmaceutical injection formulations.

In this paper we describe the validation of a capillary GC assay method for the determination of trace quantities of benzaldehyde in several drug formulations that contain benzyl alcohol as a preservative.

2. Experimental

2.1. Instrumentation

The method was developed on a Shimadzu gas chromatograph Model GC-15A (Shimadzu, Kyoto, Japan) equipped with a flame-ionization detector and a model GC-R4A Chromatopac integrating recorder. The column used was a 25 m × 0.53 mm i.d. fused silica capillary column coated with 0.5 mm film of OV-101. The column, injector and detector temperatures were 160, 270 and 270 °C, respectively. The carrier gas was extra pure helium at a flow rate of 4.5 ml/min. The injection volume was 1 μl and the samples were injected in the split-less mode with a Hamilton syringe.

2.2. Materials

All chemicals were of analytical reagent grade. Benzyl alcohol, benzaldehyde, 3-chlorobenzaldehyde and chloroform were obtained from Merck, Darmstadt, Germany.

Various batches of generic Na-diclofenac, piroxicam and Vitamin B-complex injection solutions were kindly supplied by the following manufacturers in Iran (the letter in parentheses is used to designate the manufacturer in the assay results): Chimidaru (A), Alborzdaru (B), Zahravi (C), LPC (D), and Osvah (E).

The following injection solutions were used as reference formulations: Voltaren®, [Novartis Pharma GmbH, Germany (F)], Feldene®, [Pfizer, Spain (G)] and Becozyme® [Roche, Switzerland (H)].

2.3. Preparation of stock and standard solutions

Stock solutions of benzaldehyde, benzyl alcohol and 3-chlorobenzaldehyde (used as internal standard) were prepared by dissolving separately 50 mg of each (accurately weighed) in 50 ml chloroform. These solutions were protected from light and were used on the day of preparation. Subsequent working standards were prepared in chloroform from stock solutions by varying the concentration of benzaldehyde between 0.5 and

100 μg/ml and maintaining the internal standard at a constant level of 100 μg/ml.

2.4. Calibration curves

Triplicate 1 μl injections were made for each concentration and the peak area ratio of benzaldehyde to the internal standard (3-chlorobenzaldehyde) was plotted against the corresponding concentration to obtain the calibration graph.

2.5. Pharmaceutical preparation

Each commercial injection dosage form of Na-diclofenac has a volume of 3 ml and contains usually 40–50 mg/ml benzyl alcohol, while the piroxicam is 1 ml and has a content of 20 mg benzyl alcohol per dosage form. The nominal benzyl alcohol concentration of commercial pharmaceutical vitamin B-complex injection solution (2 ml) is 10 mg/ml.

For each formulation, the contents of ten ampoules of the parenteral dosage form were poured into the separating funnel. To each funnel was added a calculated amount of internal standard solution in chloroform, so that the final concentration of 3-chlorobenzaldehyde was 100 μg/ml. The solution was mixed thoroughly and then successively extracted three times with an equal volume of chloroform for 3 min. The chloroform extracts were combined and dried over anhydrous sodium sulfate. Under the chromatographic conditions described, triplicate 1 μl injections of each chloroform extract were made for analysis.

2.6. Recovery studies

Appropriate known quantities of benzaldehyde and internal standard were added to each injection sample (Na-diclofenac, Piroxicam and Vitamin B-Complex) and mixed thoroughly. Each spiked sample was then extracted with chloroform as described above. Triplicate injections of these spiked sample extracts and the standard extracts were made in the GC. Comparisons of the integrated area ratios of peaks resulting from the injected reference standard extracts with those resulting from the spiked extracts were used to calculate the percentage recovery of benzaldehyde in each case.

3. Results and discussion

A typical gas chromatogram obtained from the analysis of a mixture of benzyl alcohol, benzaldehyde and 3-chlorobenzaldehyde (internal standard) dissolved in chloroform is shown in Fig. 1. Benzaldehyde, benzyl alcohol and the internal standard were well resolved, with retention times of 3.648, 3.948 and 4.613 min, respectively.

In Fig. 2 are typical chromatograms showing the separation of benzaldehyde, benzyl alcohol and internal standard in the studied injection formulation samples, obtained after addition of internal standard and extraction into chloroform. Effective

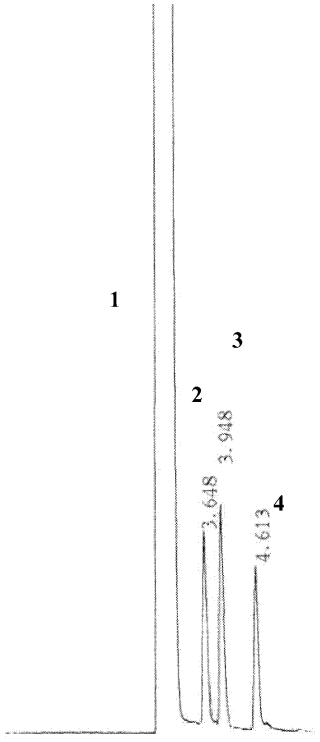

Fig. 1. Gas Chromatogram of a standard mixture of [2] Benzyl alcohol, [3] Benzaldehyde and [4] 3-Chlorobenzaldehyde (internal standard) in [1] chloroform. Retention times in minutes are shown.

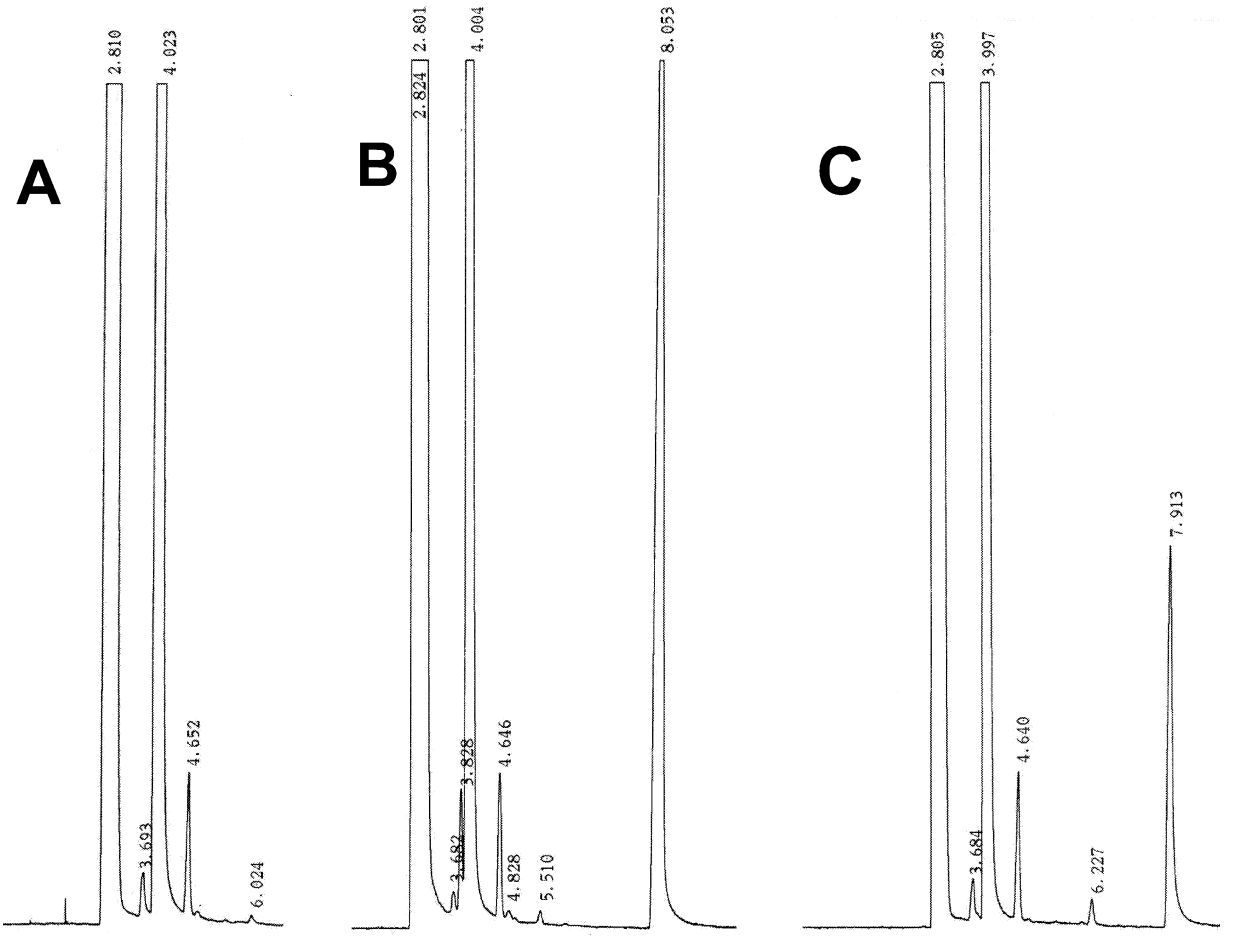

Fig. 2. Gas Chromatograms of chloroform extracts of [A] Voltaren, [B] Piroxicam and [C] Becozyme. Peak identification as in Fig. 1.

benzaldehyde extraction from the injection formulations is the key to success of this assay. Chloroform was selected as the extracting solvent, because benzaldehyde is extracted to better than 95% completion with three portions of chloroform, without any observed co-extraction of interfering components from the injection formulations. The chromatograms shown in Fig. 2 clearly demonstrate the advantage of this solvent and the disappearance of other component peaks when compared with other solvents. The GC conditions described gave complete elution with baseline resolution of benzaldehyde, benzyl alcohol and internal standard within a run time of less than 7 min.

Validation of this GC method was performed to establish the repeatability, recovery and specificity of the assay, and to determine the detector linearity and limit of detection (LOD) of benzaldehyde.

3.1. Validation

3.1.1. Calibration graph linearity

The calibration graph for peak area ratio against the concentration ratio of benzaldehyde to internal standard was constructed using the stated conditions. The ratios showed a linear response for the range 0.5–100 μg/ml benzaldehyde at the constant concentration of 100 μg/ml

for the internal standard. The correlation coefficient (r) of the calibration curve (peak area ratio vs. concentration ratio) in this range was >0.995. The equation of this curve ($y=mx+b$) was then used to calculate the unknown benzaldehyde concentration in the studied injection formulations. The values for m (intercept) and b (slope) with the respective 95% confidence intervals were (0.0150 ± 0.0002) and ($\sim0.002\pm0.009$), respectively, with the concentration scale in μg/ml.

3.1.2. Repeatability/reproducibility

The intra- and inter-day variability of the retention times and amounts detected was performed on standard solutions containing three different concentrations of benzaldehyde with a constant concentration (100 μg/ml) of internal standard, as given in Table 1. The results show excellent accuracy, repeatability and reproducibility for the assay performed on 1 day or over 5 consecutive days. An indication of the ruggedness of the method comes from the fact that the R.S.D. values for intra- and inter-day assays of benzaldehyde in the cited formulations performed in the same laboratory by two analysts did not exceed 2.5%.

3.1.3. Analytical recovery

The reference samples of Na-diclofenac, piroxicam and B-complex injection formulations were found to contain detectable amounts of benzaldehyde. Therefore, recovery assessment was performed by analyzing reference samples that were spiked with a known amount of benzaldehyde (proportional to the amount of benzyl alcohol used in each formulation) and 100 μg/ml internal standard. The total recovery of benzaldehyde from these spiked injection formulations was $>95\%$ in each case as shown in Table 2.

3.1.4. Selectivity

The selectivity of the method was checked by monitoring standard solutions of benzaldehyde in the presence of formulation components of the Na-diclofenac, piroxicam and B-complex injection formulations separately. The ability of the system to resolve benzaldehyde from potentially interfering components and internal standard was good. The responses were not different from that obtained in the calibration. Hence, the determination of benzaldehyde in these formulations is considered to be free from interference due to formulation components.

3.1.5. Sensitivity

The sensitivity of the method can be determined through the limit of quantitation (LOQ), and LOD. These limits for benzaldehyde were measured by injection of 1 μl of standard solutions successively diluted. Thus the LOQ and LOD were found to be 0.4 μg/ml (signal-to-noise ratio 10:1) and 0.1 μg/ml (signal-to-noise ratio 3:1), respectively.

3.1.6. Pharmaceutical application

The proposed method was extended to the determination of benzaldehyde in several different pharmaceutical injection dosage forms that use benzyl alcohol as preservative. The analytical results summarized in Table 3 indicate that the

Table 1
Intra- and inter-day precision of GC determination of benzaldehyde standard solutions

Benzaldehyde standard (μg/ml)	Retention time		Benzaldehyde concentration measured[a]			
	Intra- and inter-day[a]		Intra-day		Inter-day	
	Mean (min)	±S.D.	Mean (μg/ml)	R.S.D. (%)	Mean (μg/ml)	R.S.D. (%)
15	3.64	0.23	14.8	1.1	14.6	1.3
30	3.67	0.18	29.9	0.88	29.8	0.92
45	3.65	0.20	44.8	0.97	29.6	1.2

[a] Mean value for three different standards for each concentration. Inter-day reproducibility was determined from three different runs of fresh prepared standard solutions over 5 consecutive days.

Table 2
Determination of benzaldehyde in injection dosage forms containing added benzaldehyde

Injection formulation	Benzaldehyde found (μg/ml)[a]	Content added (μg/ml)	Total benzaldehyde determined (μg/ml)[a]	Recovery (%)	R.S.D. (%)
Voltaren®	11.5	29.0	39.6	97.8	1.4
Feldene®	2.7	18.2	20.5	98.1	1.9
Becozyme®	11.2	10.4	20.6	95.2	2.0

[a] Average of three determinations.

method does not suffer any interference from common components used in these injections, such as the active compounds, benzyl alcohol, Na-metabisulfite, propylene glycol, nicotinamide, or buffer components. In particular, the presence of Na-metabisulfite in Na-diclofenac injections presents a real possibility of affecting the concentration of benzaldehyde determined because of the classical addition of the nucleophile to the carbonyl system producing a sulfonic compound that is either chloroform-insoluble or not detectable by GC [11].

Some injection formulations, especially the generic brands of Na-diclofenac injection, were found to contain very high levels of the neurotoxic benzaldehyde (ca. proportional to the amount of benzyl alcohol used in the formulations). These were some examples of the formulations associated with the occurrence of paraplegia and other adverse neurotoxic side effects.

4. Conclusion

The GLC method proposed for selective quantitation of neurotoxic benzaldehyde is suitable for application to the quality control analysis of benzyl alcohol containing injection formulations such as Na-diclofenac, Piroxicam and Vitamin B-complex. The extraction procedure is straightforward and a moderate analysis time is achieved at the concentration levels involved (1.0–100 μg/ml) without the need for solvent evaporation. The method validation demonstrated good precision, specificity and accuracy with acceptable recovery and chromatographic resolution. The level of

Table 3
Determination of benzaldehyde in commercial injection formulations using the GC procedure

Drug product (manufacturer code)	Benzyl alcohol label claim (mg per injection)	Amount of benzaldehyde found[a]		R.S.D. (%)
		μg per injection	As % of benzyl alcohol	
Voltaren (F)	120	34.5	0.029	1.2
Na-diclofenac (A)	120	334.8	0.279	0.8
Na-diclofenac (B)	120	225.3	0.188	0.9
Na-diclofenac (C)	141	451.5	0.320	0.5
Feldene (G)	20	2.7	0.014	1.6
Piroxicam (D)	20	16.9	0.085	1.0
Piroxicam (E)	20	19.2	0.096	0.8
Becozyme (H)	20	22.4	0.112	1.8
B-Complex (C)	20	36.8	0.184	0.7
B-Complex (E)	18	33.0	0.183	0.9

[a] Average of three determinations.

precision is suitable for the routine control analysis of benzaldehyde in pharmaceutical injection formulations. The LOQ, defined as the lowest concentration in the calibration curve that could be determined with acceptable precision and accuracy, was 0.4 μg/ml. Thus the sensitivity of the proposed method is more than sufficient for benzaldehyde assay in injection formulations that use benzyl alcohol as preservative.

Only the reference formulations contained an amount of benzaldehyde that was less than the maximum permitted by the British and European Pharmacopoeias, i.e. 0.05% of the benzyl alcohol content. All of the generic formulations exceeded the benzaldehyde limit by significant amounts, particularly the diclofenac formulations. Indeed, the nature of the findings reported here serves to illustrate the need for stringent quality control of the materials used in preparation of these injection formulations. In particular, the following steps have been recommended [11,12]. Either, the replacement of benzyl alcohol by another preservative or co-solvent has been suggested by the US FDA, or a decrease in the benzyl alcohol concentration together with the use of especially pure benzyl alcohol for injection. Most importantly for the latter option must be the avoidance of heat sterilization and exposure to light.

Acknowledgements

The authors wish to thank Dr S. Parvizi for his invaluable help in the provision of reference and product injection formulation samples.

References

[1] A.G. Kazemifard, D.E. Moore, A. Mohammadi, J. Pharm. Biomed. Anal. 30 (2002) 257–262.
[2] J.H. Saiki, S. Thompson, F. Smith, R. Atkinson, Cancer 29 (1972) 370–374.
[3] A.F. Hahn, T.E. Feasby, J.J. Gilbert, Neurology 33 (1983) 1032–1038.
[4] D. Duncan, W.H. Jarvis, Anesthesiology 4 (1943) 465–474.
[5] The United States Pharmacopoeia 24 and The National Formulary 19, Rockville, MD, USA, 2000, p. 2420.
[6] The British Pharmacopoeia, HMSO, London, 2001, p. 204.
[7] A.P. Litovka, M.N. Manakov, Khim. Khim.Tekhnd. 16 (1973) 1227–1229.
[8] P. Yue, Z. Zhang, Yao Wu Fen Hsi Tsa Chih 1 (1981) 335–339.
[9] G.A. Zennina, A.P. Veronin, Gigiena Trudai Professionanye Zabolevaniia 5 (1985) 55–56.
[10] I.I. Hewala, J. Pharm. Biomed. Anal. 12 (1994) 73–79.
[11] I.I. Hewala, Talanta 40 (1993) 919–923.
[12] A.H. Kibbe, Handbook of Pharmaceutical Excipients, third ed., American Pharmaceutical Association and Pharmaceutical Press, Washington DC, 2000, p. 41.