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Research Article

HPLC – UV CHROMATOGRAPHY DETERMINATION OF BENZALDEHYDE ARISING FROM BENZYL ALCOHOL USED AS PRESERVATIVE IN INJECTABLE FORMULATIONS.

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ABSTRACT

A simple, rapid, precise and accurate HPLC – UV procedure has been developed to determine benzaldehyde, the toxic oxidation product of the widely used preservative and Co – solvent, benzyl alcohol, in injectable formulations. Benzaldehyde as a main potential impurity of benzyl alcohol may cause many toxic effects such as neurotoxicity, hypersensitivity in human and fatal toxic syndrome in premature infants. Hence, detection and quantification of low level of benzaldehyde in injectable preparations containing benzyl alcohol is necessary. There is Capillary electrophoresis and Gas chromatographic methods for detection and quantification of benzaldehyde but this method are non – compendial and not cost beneficial. The presented HPLC method is rapid and simple in comparison. Separation and quantification are achieved isocratically on Novapak C18 column (250 mm ×4.5 mm, 5 µm i.d.), and UV detection at 254 nm. Equal mixture of acetonitrile and water at a flow rate of 1.2 ml/min was used as mobile phase.

Keywords: Benzyl alcohol, Benzaldehyde, Determination, Injectable formulation, HPLC-UV Chromatography

INTRODUCTION

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

The United States Pharmacopoeia limits the presence of benzaldehyde in benzyl alcohol to levels of 0.15%, with quantification by GC [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 Capillary electrophoresis and Gas chromatographic methods for detection and quantification of benzaldehyde are not cost beneficial [7-11]. In this paper the validation of a HPLC assay method was developed for the determination of trace quantities of benzaldehyde in Midazolam injections that contain benzyl alcohol as a preservative. .

In this study a rapid and sensitive high performance liquid chromatographic method capable of quantifying benzaldehyde to 50ng/ml in Midazolam injections was developed.

MATERIALS AND METHODS

Materials

All chemicals reagent were HPLC grade from Merck, Darmstadt. Benzaldehyde was obtained from Merck, Darmstadt, Germany. Purified HPLC grade water was prepared by reverse osmosis and filtration through a Milli-Q® system (Millipore, Milford, MA, USA). Midazolam working standard powder was kindly provided by Profarmaco Italy. Various batches of generic Midazolam injection solutions were supplied by Tehran Chemie Pharmaceutical Company, Tehran, Iran.

Sample and standard solution preparation

In this study, Stock standard solutions of benzaldehyde, was prepared by dissolving 10 mg of benzaldehyde (accurately weighed) in 100 ml of mobile phase.

These solutions were protected from light and were used on the day of preparation. subsequent working standards were prepared in mobile phase from stock solutions by varying the concentration of benzaldehyde between 5 µg/ml and 30 µg/ml. (5 µg/ml ,10 µg/ml,15 µg/ml,20 µg/ml,25 µg/ml and 30 µg/ml)

Resolution solution was prepared in mobile phase from stock standard solution of benzaldehyd and stock solution of benzyl alcohol. In final resolution solution, concentration of benzyl alcohol and benzaldehyd was 20 µg/ml and 5 µg/ml, respectively.

Each injection dosage form of Midazolam has a volume of 5mg/1ml and contains usually 8-12 mg/ml benzyl alcohol. For preparation of test solution, 10 ml of Midazolm injections was diluted by using 100ml of mobile phase

Chromatographic system and conditions

The HPLC system consisted of a Younglin® 930D controller solvent delivery module (Younglin instrument, Korea), including quaternary pump, mobile phase degasser, manual Rheodyne® injection system, coupled with a Younglin ®730D detector. Waters ® Novapak C18 (250 mm× 4.6 mm, 4µm) analytical columns were used for method development and validation.

The Autochroo®3000 chromatographic software was used for data acquisition and processing. The mobile phase consisted of a mixture of water and Acetonitrile. (50:50, v/v%).

During assay, an aliquot of 20 µl of diluted samples were injected in duplicate into the analytical column at a flow rate of 1.2 ml/min. Benzaldehyde was detected at 254nm. The analytical column, theoretical plate number and tailing factor of the analytes under different chromatographic conditions were calculated using USP methods [12].

RESULTS AND DISCUSSION

Method development

In this study, the mobile phase ratio and UV wavelength were adjusted to achieve the best retention time and peak resolution between benzaldehyd and benzyl alcohol. (R= 5.32)

This result is presented in Fig. 1.

Calibration curves and graph linearity

Triplicate 20 µl injections were made for each concentration and the peak area ratio of benzaldehyde was plotted against the corresponding concentration to obtain the calibration graph.

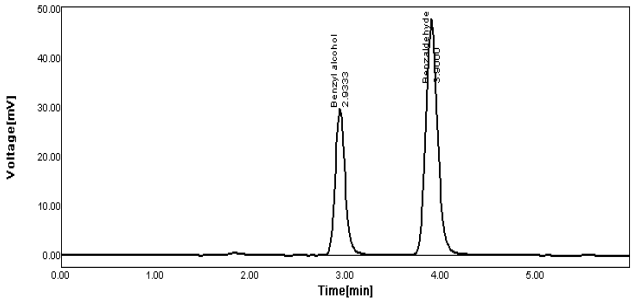


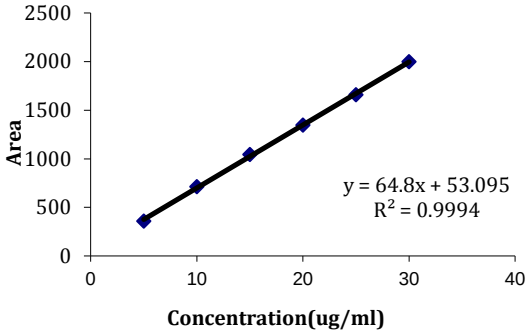
Fig. 1: Chromatogram of resolution solution

The calibration graph for peak area ratio against the concentration ratio of benzaldehyde was constructed using the stated conditions. The linearity of this method was determined at six concentration levels from 5-30µg/ml.

The ratios showed a linear response for the range 5-30µg/ml benzaldehyde. The correlation coefficient (r^2) of the calibration curve (peak area ratio vs. concentration ratio) in this range was found to be 0.9994. The equation of this curve (y= mx+b) was used to calculate the unknown benzaldehyde concentration in the studied injection formulations.

The regression equation was found to be y=64.8 x +53.095.

In the Fig. 2 the calibration curve is demonstrated the level of benzaldehyde.



Specificity

The specificity of the method was checked by monitoring standard solutions of benzaldehyde in the presence of formulation components of the Midazolam injection formulations. The ability of the system to resolve benzaldehyde from potentially interfering components was acceptable.

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. The specificity of the HPLC method is illustrated in Fig. 3.

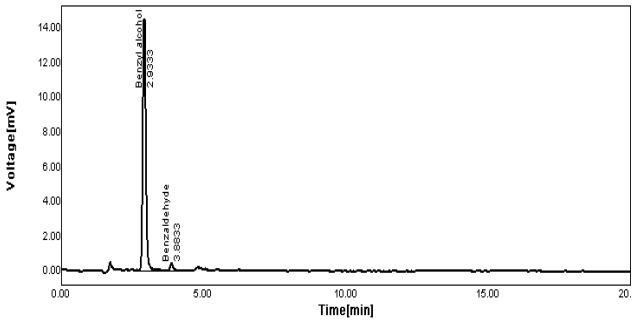


Fig. 3: Specificity of benzaldehyde.

Precision

The precision of an analytical procedure is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple sampling of homogeneous sample.

The precision of an analytical procedure is usually expressed as the SD or RSD of series of measurement. The ICH documents recommend that repeatability should be assessed using a minimum of nine determination at covering the specified range for procedure (i.e. three concentrations and three replicates of each concentration) [13].

Repeatability was investigated by injection three replicate samples of each of the 10, 20 and 30 µg / ml standards solution.

Precision data for benzaldehyde was shown in Table 1.

This indicated that method was highly precise.

Table 1: Result of precision (n=3)

S.No.	Concentration(µg/ml)	Mean Area	SD	CV%
1.	10	714.05	4.46	0.62
2.	20	1355.33	9.85	0.72
3.	30	2002.25	12.25	0.61

Analytical recovery

Appropriate known quantities of benzaldehyde were added to each injection sample and mixed thoroughly. Three different levels of standard (corresponding to 80% to 120% from 20-30 µg/ml) were added to the previously analyzed samples .Triplicate injections of this spiked sample and the standard were made in HPLC. Comparisons of the integrated area ratios of peaks resulting from the injected standard with those resulting from the spiked samples were used to calculate the percentage recovery of benzaldehyde in each case.

The reference samples of Midazolam injection formulations were found to contain detectable amounts of benzaldehyde. The result of recovery studies shown in Table 2.

Table 2: Result of Recovery (n=3)

Concentration (µg/ml)	%Recovery (1)	%Recovery (2)	%Recovery (3)	%Recovery (Average)	RSD %
20	99.8	101.46	102.83	101.36	1.5
25	100.26	97.65	100.55	99.48	1.6
30	97.74	97.71	101.67	99.04	2.3
			Average	99.96	1.23

The total recovery of benzaldehyde from these spiked injection formulations were 99.96%.

Sensitivity

The sensitivity of the method can be determined through the limit of quantitation (LOQ), and LOD. These limits for benzaldehyde were determined based on signal-to-noise ratios and were determine using an analytical responses of 10 and 3 times the background noise, respectively [14].

Thus the LOQ and LOD were found to be 50ng/ml (signal-to-noise ratio 10) and 10ng/ml (signal-to-noise ratio 3), respectively.

CONCLUSION

The HPLC method proposed for selective quantitation of neurotoxic benzaldehyde is suitable for application to the quality control analysis of benzyl alcohol containing Midazolam injections. The method validation demonstrated good precision, specificity and accuracy with acceptable recovery and chromatographic resolution. The level of precision is suitable for the routine control analysis of benzaldehyde in pharmaceutical injection formulation .The results of the analytical validation parameters are summarized in Table 3.

Thus the sensitivity of the proposed method is more than sufficient for benzaldehyde assay in injections formulations that use benzyl alcohol as preservative.

Table 3: Summarized validation results

Parameter	Acceptance criteria	Results
Linearity	R ≥ 0.99	0.9994
Accuracy	Recovery : 98%-102%	99.96%
Precision	C V ≤ 1 %	0.65%
Specificity	Resolution ≥ 2	5.32
	No peak about 3.9 minute	Conforms
System suitability	Tailing Factor ≤ 2	1.011
	Theoretical Plates > 2000	4654

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.

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 [15, 16]. 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.

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References

1. Kazemifard AG, Moore DE, Mohammadi A. Polarographic determination of benzaldehyde in benzyl alcohol and sodium diclofenac injection formulations. J Pharm Biomed Anal 2002; 30:257_ 62.
2. Saiki J H, Thompson S., Smith F and Atkinson R. Paraplegia following intrathecal chemotherapy, Cancer 1972;29 : 370.
3. Hahn AF; Feasby T E; Gilbert J J. Paraparesis following intrathecal chemotherapy Neurology. 1983. Aug; 33(8):1032-8.
4. Duncan D and Jarvis W H. Anesthesiology. 1943; 4: 465.
5. The United States Pharmacopoeia 32 and The National Formulary 27, Rockville, MD: USA; 2009, 2420.
6. The British Pharmacopoeia, HMSO Publication: London; 2010, 245.
7. Litovka AP, Manakov MN.Analysis of Anhydrides on Monocarboxylic Acids by Gas-Liquid chromatography.Tekhnol 1973;16 (8);1227_ 29.
8. Yue P, Zhang Z. Analysis for benzyl alcohol and its oxidation product [benzaldehyde] in erythromycin lactobionate injections by infra-red spectrophotometry and gas chromatography.
9. Zennina GA, Veronin AP. Gas chromatographic determination of benzyl alcohol, benzaldehyde and benzyl acetate in the air. Gigiena Trudai Professionanye Zabolevaniia 1985; 5:55_ 56.
10. Hewala I I. Gas-liquid chromatographic and difference spectrophotometric techniques for the determination of benzaldehyde in benzyl alcohol.J Pharm Biomed Anal 1994 ; 12 :73_ 79.
11. Kazemifard AG, Moore DE, Mohammadi A and Kebriyaeezadeh A. Capillary gas chromatography determination of benzaldehyde arising from benzyl alcohol used as preservative in injectable formulations .J Pharm Biomed Anal 2003;31:685-91
12. The United States Pharmacopoeia 32 and The National Formulary 27, Rockville, MD:USA;2009,. 235.
13. ICH draft Guidelines on validation of Analytical procedures .Definitions and Terminology,Federal Register ,IFPMA,Switzerland 1995,vol.60.
14. The United States Pharmacopoeia 32 and The National Formulary 27, Rockville, MD:USA;2009, 735.
15. Hewala I I. Difference spectrophotometric assay of benzaldehyde in benzyl alcohol. Talanta 1993;. 40:919_ 23.
16. Kibbe AH.Handbook of Pharmaceutical Excipients, third ed., American Pharmaceutical Association and Pharmaceutical Press, Washington DC 2000; 41.