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A rapid spectrophotometric method for estimation of organic volatile impurity in hepatoprotective marketed formulations

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Abstract

Organic volatile impurities are chemicals that are used at different stages of synthesis, extraction, isolation and purification of active pharmaceutical ingredients, excipients and drug products. Since the residual solvents add no value to the quality and stability of the drugs and drug products. Therefore, they should be removed in order to meet product specifications and other quality based requirements. Moreover, some of these solvents possess established carcinogenic properties and may enter the formulation as residual solvents. These residual solvents cannot be removed completely, hence they should be within the acceptance limits as per the regulatory guidelines such as ICH guidelines (Q3C). There has been a lot of hue and cry that formulations available in the market are not properly standardized for their quality due to lack of stringent regulations and standards from regulatory authorities. The aim of the present work was to develop a simple, accurate and specific UV method for the determination of ethanol as residual solvent in marketed formulations. The developed method was validated as per ICH guidelines and all the parameters are found to be within the limits.

Keywords: Ethanol, quantitative determination, residual solvent, marketed formulations

Introduction

The liver is one of the largest organ in the body and it plays important role in regulation of physiological processes. One of the major functions of liver is to detoxify and metabolize chemicals/drugs in the body. Hepatoprotective drugs may protect the liver by several mechanisms such as elimination of virus, blockage of fibrogenesis, inhibition of oxidative injury and

suppression of tumorigenesis. The drugs in liver are metabolized mainly by oxidation, reduction, hydrolysis, hydration, conjugation, condensation and isomerization. The purpose of metabolism is to make the excretion of the drug easier. Enzymes involved in metabolism are present in many tissues but generally more concentrated in the liver. Organic volatile impurities (OVIs), commonly referred to as residual solvents, are organic volatile chemicals used or produced in the manufacturing of drug substances and excipients, or in the preparation of drug products. In addition, it is well known that during preparation of active pharmaceutical ingredients, excipients and drug products many solvents viz methanol, ethanol, isopropyl alcohol, acetonitrile, acetone, toluene, butanol, hexane, cyclohexane, heptanes, dichloromethane etc. are used for synthesis, extraction, isolation and purification in different stages of development. These solvents cannot be completely removed by practical process such as freeze drying and drying at higher temperature under vacuum (1-4). The residue of solvents that remain with the formulations is referred as residual solvents or OVIs that have no therapeutic benefits and are toxic and hazardous to human health. These OVIs are likely to be carried in the marketed formulations too. In addition, these solvents may irritate and damage the skin, eyes, respiratory tract, cause a narcotic effect on the nervous system, and damage internal organs such as the liver and kidneys by acute (from single heavy exposures) or chronic (from repeated low dose exposures over months or years). Some solvents may even cause specific diseases such as cancer. Even more dangerous condition is when such types of OVIs are present in hepatic formulations. As discussed earlier, metabolism of drugs occurs in liver and marketed formulations with organic volatile impurities may produce unwanted side effects to the users/patients (5-9).

The present work illustrates the simple and rapid method for determination of ethanol in marketed formulations. This method demonstrates solvent extraction of ethanol from formulations followed by measurement of alcohol by spectrophotometer using acid dichromate solution. Determination of ethanol based on oxidation of ethanol by reacting with excess of acidic potassium dichoromate solution. When ethanol is present in an aqueous solution, chromium ions oxidize ethanol, and these ions are reduced from the +6 oxidation state to +3, changing the color from orange to green (10-13). Various techniques have been developed to determine ethanol concentrations in different solutions but an ideal method for this purpose should be rapid, cheap, simple, accurate, sensitive and reproducible. Furthermore, the method that does not require expensive instrument and well-trained personnel is primarily preferred. Gas chromatography (GC) is one of the common method for determination of ethanol concentration due to its accuracy, and sensitivity. However, this method requires expensive instrument and skilled worker for analysis (14). So the main aim of present work was to determine organic volatile impurity namely ethanol in marketed formulations by developing and validating a simple, rapid, economic, sensitive and accurate method using UV spectrophotometer.

Materials and Methods

Chemicals and Reagents: All the solvents and reagents used in the study were of AR/GR grade and purchased from different companies as Qualikems,

Finar and Merck. The commercial formulations were procured from the local market.

Instrumentation

The analysis was carried out on BioPhotometer D30, 230 V/50 – 60 Hz (Eppendorf) equipped with 1cm quartz cells.

Extraction and Preparation of Solution

Tri-*n*-butyl phosphate (TBP) was selected as a solvent for extraction of ethanol from formulations. The required concentration of sample was diluted with equal fraction of distilled water and mixed with 5 ml TBP by vigorous vortexing for 20 minutes. Then tubes were kept aside for phase separation and upper layer was used for dichromate oxidation. Standard solutions of ethanol were prepared by diluting specific amount of absolute alcohol using distilled water and processed similarly (10-12).

Preparation of dichromate reagent

The dichromate reagent required for study was prepared by dissolving 10g potassium dichromate in approximately 20ml of distilled water. Then 70ml concentrated H_2SO_4 was added cautiously, the resultant solution was cooled and the volume was adjusted to 100ml by adding sufficient volume of distilled water (10-12).

Dichromate oxidation and spectrophotometric analysis

5ml of TBP layer was transferred to new tube and mixed with 5 ml of dichromate reagent by shaking at 250rpm for 20 minutes. Afterward, lower layer was separated and subjected to measurement of absorbance at 590nm using spectrophotometer (10-12).

Method Validation

The method was validated by evaluating linearity, range, specificity, accuracy, precision, robustness, ruggedness, limit of detection (LOD) and limit of quantitation (LOQ) in accordance with the ICH guidelines (15-21).

Results and Discussion

UV-visible spectrophotometry is an analytical technique widely used in the quality control of drugs, as also described in official monographs, for identification and quantitation. Although there are limitations regarding specificity, the UV spectroscopy presents some advantages when compared to chromatographic methods, such as faster analysis, low operating cost and low generation of waste. An alternative for improving the specificity and sensitivity of the technique is to perform UV derivative spectrophotometric method. The method discussed in the present work provides a convenient, precise and accurate way for estimation of ethanol in marketed dosage forms (14). Absorbance maxima of ethanol at 590nm were selected for the analysis. The linear regression equations for ethanol was found to be y = 0.0021x – 0.0012. The regression coefficient value (r^2) was found to be 0.9998 indicating a high degree of linearity. The results showed an excellent correlation between the absorbance and concentration of ethanol within the selected concentration range of 2-256 μg mL^{-1}. The results confirmed the linearity and the reproducibility of the method. The *f*-test and Student's *t*-test ($P < 0.05$) confirmed insignificant difference in the predicted and observed values. The linearity curve of ethanol is given in Figure 1 and linearity parameters for the ethanol are given Table 1. The specificity studies revealed the absence of any other solvent interference in the developed method. The intra-day and inter-day precisions were assessed by analyzing standard solutions. The % RSD (relative standard deviation) was

found to be 0.62 and 1.47 for intra-day and inter-day precisions respectively. The low value of %RSD showed that the method is precise within the acceptance limit of 2%. The intra- and inter-day variability or precision data are given in Table 2. The results obtained from the intra- and inter-day were evaluated statistically using the *f*-test and Student's *t*-test. The calculated value of *f*-test and Student's *t*-test indicated that the intra- and inter-day data did not differ significantly in terms of precision. Recovery study was carried out using standard addition method at three different levels of 50%, 100% and 150%. Three samples were prepared for each recovery level. The solutions were analyzed and the percentage recoveries were calculated from the calibration curve. % recovery for ethanol was found in the range of 99.94% and 101.05% (Table 3). The LOD and LOQ predict the sensitivity of the method. The LOD for ethanol was found to be 0.88 µg mL^{-1}, whereas LOQ were noted to be 1.97 µg mL^{-1}. The values indicated that the method is sensitive. Based on the results obtained, it is found that the proposed method is accurate, precise, reproducible & economical and can be employed for routine quality control of ethanol pharmaceutical dosage form. Analysis of marketed hepatic formulations (Hepamerz, Reheptin, Usrocol 150, Alcoliv, Liv-52, Silybon 150, Creon 10000, Sifaliv, and Culiv) was carried out using proposed method. In this study, codes (F_01 to F_09) were used randomly for abovementioned hepatic marketed formulations. The results are presented in ppm (parts per million) and summarized in the Table 4.

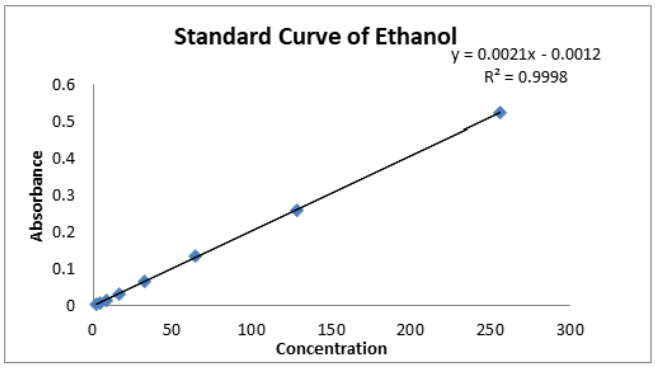

Figure 1: Calibration curve of ethanol

Table 1: Linearity parameters for ethanol

Linearity Parameter	**Ethanol**
Range	2-256 µg mL^{-1}
Slope	0.0021
Intercept	-0.0012
Regression coefficient (r^2)	0.9998
f-test	1.77 (6.09)[a]
t-test	0.25 (2.21)[a]

[a]The values in parenthesis are the theoretical values of *f*-test and student's *t*-test at 95% confidence level.

Table 2: Statistical treatment of the precision data

Parameter	Ethanol
Intraday or Repeatability (%RSD)	0.62
Inter day (%RSD)	1.47
f-test	3.84 (6.82)[a]
t-test	1.37 (2.89)[a]

[a]The values in parenthesis are the theoretical values of *f*-test and student's *t*-test at 95% confidence level.

Table 3: Percent recovery data

% simulated dosage nominal	% Mean (n=3)	±SD	RSD (%)
50	100.25	0.90	0.84
100	99.94	1.31	1.12
150	101.05	1.49	1.26

Table 4: Analysis of marketed hepatic formulations

Marketed formulation	Ethanol (in ppm)
ICH permissible limits (in ppm)	5000
F_01	ND
F_02	811
F_03	ND
F_04	ND
F_05	772
F_06	1268
F_07	ND
F_08	1477
F_09	0

ND = Not detected

Conclusion

Solvents are likely to make their entry in finished marketed formulations due to their varied use in a number of steps during different stages of development and preparation. The solvent residues may deteriorate/reduce efficacy and stability of such formulations and may prove to be a potential risk to human health. Therefore, the determination of residual solvents is an important issue. This study on hepatic marketed formulations reflects that no ethanol was detected in some of the formulations, however if present, were within prescribed permissible limits of ICH guidelines. The study also presents a simple, specific, accurate, precise and rugged spectrophotometric method for estimation of ethanol. In addition, dichromate oxidation is a consistent and reliable method for quantitative determination of ethanol in formulations.

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