Andonova et al., J Pharm Sci Emerg Drugs 2020, 8:4
DOI: 10.37532/jpsed.2020.8(4).143

Journal of Pharmaceutical Sciences & Emerging Drugs

Research Article

A SCITECHNOL JOURNAL

Development and Validation of HPLC/DAD Method for Analysis of Erufosine

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Received date: June 26, 2020; **Accepted date:** July 10, 2020; **Published date:** July 16, 2020

Abstract

Objective: The aim of this study is to develop, validate and apply a high-performance liquid chromatography method with diode array detection (HPLC/DAD) for identification and quantification of anti-cancer agent Erufosine (erucylphospho-N,N,N-trimethylpropylammonium, ErPC3) which is the most recent and promising member of the alkyl phosphorylcholine family.

Methods: HPLC/DAD method with isocratic elution using column C18 ODS, 250 × 4.6, 100 A, 5 µm, mobile phase Methanol/DI Water (85:15 v/v), flow rate 1.0 ml/min and UV detection at 206 nm.

Results: The method was developed and validated in respect of analytical and chromatographic parameters specificity, repeatability, accuracy, limit of detection, limit of quantitation, linearity, system suitability test and robustness. The method is characterized by good reproducibility, accuracy and linearity in the specified concentration range of 1.0 µg – 1.0 mg.

Conclusion: A fast, simple, accurate, precise, and linear HPLC method has been developed and validated for the simultaneous analysis of anti-cancer agent erufosine in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and European Pharmacopoeia 9.0 requirements. The method is of practical importance for development of identification and assay tests of Esufosune and also it could be used during the phase of clinical trials.

Keywords: Erufosine; Alkyl phosphorylholines; Anti-cancer agents; HPLC/DAD method; Validation

Introduction

Alkyl Phosphorylcholines (APCs) are promising class of anti-neoplastic agents exhibiting significant cytotoxic and proapoptotic activity towards a vast number of tumour cell lines[1-3]. An important fact is that APCs does not manifest myelotoxicity. They even stimulate normal haematopoiesis [4-9]. Thus they could be used in combination treatment regimens aiming to ameliorate the toxicity of conventional anti-neoplastic agents. APCs increase the cytotoxicity of various conventional cytotoxic drugs [7-11] and sensitize tumour cells to radiation-induced cell death [12]. Unlike to standard chemotherapeutic drugs, APCs does not interact directly with cellular DNA. They target cellular membranes and interfere with membrane lipid composition and the formation of lipid second messengers. Thereby the synthetic phospholipid derivatives affect the growth, cell cycle progression and survival of tumor cells [13-15].

The development of clinically useful ALPs (Figure 1) has started with Edelfosine which is potent immune modulator and effective inhibitor of cell proliferation showing a high degree of selectivity towards tumor cells. Ilmofosine has demonstrated both in vitro and in vivo anti-tumor activity in a variety of tumors, but lacked clinical activity in patients. However, the clinical use of both Edelfosine and Ilmofosine has remained limited due to gastrointestinal toxicity. Miltefosine exhibits effective antitumor activity but also has a hemolytic effect and today is used only in the treatment of skin metastases of breast cancer. Erufosine (erucylphospho-N,N,N-trimethylpropylammonium, ErPC3) is the most recent and promising member of the ALP family [16,17].

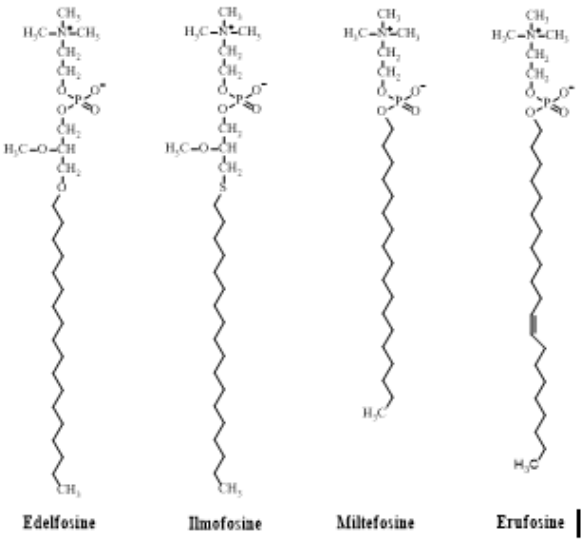

Figure 1: Chemical structures of synthetic clinically useful alkyl-phospholipids (ALPs).

Erufosine is the only derivative from this group which is suitable for intravenous administration. In aqueous solutions it forms lamellar instead of micellar structures and thus it does not manifest hemolytic and myelotoxicity [18]. Erufosine exhibits significant proapoptotic effects on many malignant cell lines [8] and primary tumor cells [19].

As a result of low toxicity and significant efficacy, Erufosine is emerging as a very promising antineoplastic agent and this requires the development of rapid, reliable and accurate tests for qualitative and quantitative analysis of drugs containing it. In the literature data there are some described HPLC methods for quantification of Miltefosine in blood samples [20,21] but lack of information about Erufosine

analysis. Furthermore Erufosine is not a pharmacopoeial substance and no methods have been developed for its analysis in research studies and clinical trials. Thus in the study presented we have developed a rapid, simple and precise analytical method using HPLC technique with isocratic elution and diode array detection. The method was validated in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and European Pharmacopoeia 9.0 requirements [22,23]. The method is robust enough to reproduce accurate and precise results under different chromatographic conditions and is of practical importance for development of identification and assay tests of Erufosine and also it could be used during the phase of clinical trials.

Materials and Methods

Reagents

All the necessary products for preparation of the mobile phase and buffers (Acetonitrile analytical grade, Methanol analytical grade, Phosphate buffer with pH=7,4 pH units) were obtained from Sigma-Aldrich (Steinheim, Germany), Deionized water (DI), Erufosine analytical standard.

Chromatographic system and conditions

The chromatographic procedure was performed with modular HPLC system UltiMate Dionex 3000 SD, Chromeleon 7.2 SR3 Systems, Thermo Fisher Scientific Inc. The separation was achieved isocratically with Column Unisol, Adela Technologies, C18 ODS, 250 × 4.6, 100 A, 5 µm eluted with three different mobile phases: Acetonitril/DI Water (50:50 v/v) (mobile phase I); Methanol/DI Water (85:15 v/v) (mobile phase II), Methanol/DI Water (80:20 v/v) (mobile phase III) and Methanol/buffer solutions with pH=7,4 (85:15 v/v) (mobile phase IV) as the mobile phase at flow rate-1.0 ml/min. Mobile phases were previously degassed by filtration through a 0.45 µm membrane filter. The detector was set up to 206 nm. The analysis was carried out at column temperature 25°C and injection volume 20 µl.

Sample preparation

Test solution (a): 50.0 mg of Erufosine analytical standard were measured and dissolved in 50.0 ml methanol to obtain a test solution (a) with concentration 1,0 mg/ml.

Test solution (b): Solution (b) of Erufosine was prepared by dilution of aliquots from test solution (a) with solvent mobile phase to obtain solution with appropriate concentration.

Results and Discussion

For analysis of Erufosine substance HPLC/DAD method was developed and validated.

Development of HPLC/DAD analytical procedure

The aim of the present work is to develop and validate a HPLC/DAD method for identification and quantification of anti-cancer agent Erufosine which is the most recent and promising member of the alkyl phosphorylcholine family. Throughout the method development several HPLC columns were selected on the basis of the chemical structure of the analyte and also on the characteristics of of the analytical columns. The best selectivity and resolution were obtained on a Unisol, Adela Technologies, and C18 ODS column. For optimisation of the mobile phases, the effect of a variety of organic modifiers such as methanol, acetonitrile, deionized water and phosphate buffers with different pH were investigated. The best results were obtained with mobile phase III-Methanol/DI Water (80: 20 v/v) (Figure 2). The selection at 206 nm was achieved measuring by normal spectrophotometry in full analytical wavelength zone from 190 to 820 nm using Multicomponent analysis software and DAD UV/VIS system.

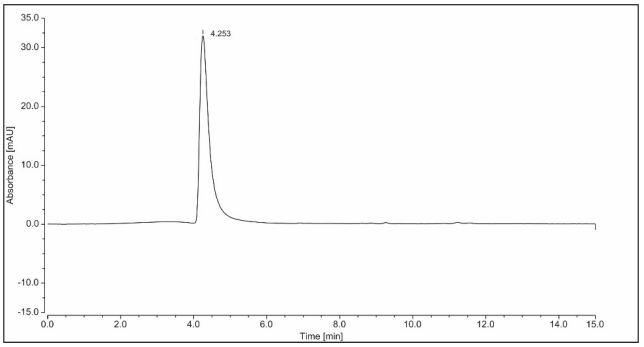

Figure 2: Chromatogram of Erufosine substance obtained with mobile phase III.

Validation of HPLC/DAD method

The elaborated HPLC method was validated in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and European Pharmacopoeia requirements.

Specificity

Specificity was determined using blank solution containing all reagents without the active substance. No significant interfering peaks (peak area >0.1%) were observed at the retention time of Erufosine in blank solution.

Repeatability

Repeatability was defined using the obtained results with mobile phase III. Therefore six equal solutions containing Erufosine with concentration 0.0001 g/ml were analyzed. Standard deviation (SD) in mAU and relative SD (RSD) in percentage were calculated. The RSD for is +/-1.5%.

Accuracy

The accuracy of an analytical procedure expresses the closeness of results obtained by that method to the true value. Accuracy was established in the specified concentration range – solutions of 50%, 100% and 150% of the amount of Erufosine were tested triplicate. The obtained results as% RSD are as follow: +/-0.76%, 0.42%, 0.88% respectively. The results of accuracy testing showed that the method is accurate within the acceptable limits.

Limit of Detection (LOD)

LOD is 1.0 μg, determined as the absolute amount of analyte that maintained a signal-to-noise ratio (peak height) of 3:1.

Limit of Quantitation (LOQ)

LOQ is 10 μg, as the absolute amount of analyte that produced a signal-to-noise ratio of 10:1. Noise was the magnitude of background response, which was determined by analyzing blank samples (mobile phase).

Linearity

Linearity was explored in concentration ratio 1.0 μg – 1.0 mg. A series of standard solutions of Erufosine across a concentrations range were prepared. The linearity of calibration curve was determined by plotting the peak area ratio of analytical standard compound to its concentration. The accordance between the absorption, measured in absorption units (AU) and concentrations in g/ml is proportional in the intervals. Using a linear regression method the slope and correlation coefficient of the linear regression equation ware determined. The correlation coefficients were found to be about 1 – 0.99084 at N=6 samples.

System suitability test

In order to perform a system suitability test the chromatographic parameters-retention time, resolution and column efficiency were determined as the number of theoretical plates to optimize the conditions. The obtained results are presented in Table 1.

Parameter	Erufosine+/-RSD (%)
RT (retention time)+/-SD in mobile phase I (min)	4.47+/-0.15
RT (retention time)+/-SD in mobile phase II (min)	4.25+/-2.47
RT (retention time)+/-SD in mobile phase III (min)	4.25+/-0.05
RT (retention time)+/-SD in mobile phase IV (min)	4.63+/-2.40
N (theoretical plates)	19913
Tailing factor	0.46
LOD (limit of detection)(μg)	1
LOQ (limit of quantitation)(μg)	10

Table 1: Results from system suitability test for Erufosine.

Robustness

The robustness of the analytical procedure is a measure of its ability to remain unaffected by small but intentional changes in the parameters of the method, analysing the samples and comparing the results to those obtained by the prescribed method and provides an indication of its reliability during normal use.

To study the robustness of the method for identification and quantification of erufosine the chromatographic behavior at four different mobile phases was monitored. The obtained results are shown in Table 2.

Mobile phase	RT (min)	Area (AU)	w (cm)	Confidence level, Error of Mean	Confidence interval, SD (%)
Acetonitrile-DI Water					
50:50 v/v (I)	4.48	78257	1	95%,	75673.6667 ±2670.858
				1.960s$\bar{x}$	(±3.53%)
	4.47	73630	1		
	4.48	75134	1		
Methanol – DI Water	4.32	242545	1.1		
85:15 v/v (II)	4.3	210775	1.2	95%,	244923.3333 ±40056.014 (±16.35%)
	4.15	281450	1.2	1.960s$\bar{x}$	
Methanol – DI Water	4.25	221645	1	95%,	220286 ±2460.71 (±1.12%)
80:20 v/v (III)	4.25	217778	0.9	1.960s$\bar{x}$	
	4.25	221435	0.9		
Methanol – phosphate buffer with pH=7.4	4.58	154096	0.7	95%,	184968.6667 ±96575.968 (±52.21%)
85:15 v/v (IV)	4.58	119357	0.8	1.960s$\bar{x}$	
	4.75	281453	1		

Table 2: Chromatographic and statistic data for erufosine at different mobile phases.

The most optimized results were observed with mobile phase III (Methanol-DI Water-80-20 v/v) and solvent methanol. Small changes of methanol concentration were reflected on the standard deviation and also deviated from the acceptance criteria [22,23]. Adding a phosphate buffer solution with pH=7.4 to mobile phase and as a solvent was leaded to obtaining of a smaller width of the peaks and also destroyed the compound.

The results obtained in the validation process determined the method as reliable and give grounds for using the developed method in tests for identity and quantification.

Conclusion

A fast, simple, accurate, precise, and linear HPLC method has been developed and validated for the simultaneous analysis of anti-cancer agent erufosine in accordance with ICH and European Pharmacopoeia 9.0 requirements. The method is of practical importance for development of identification and assay tests of Esufosune and also it could be used during the phase of clinical trials.

Acknowledgment

This work was supported by grants from Medical University – Sofia, as included in the National Scientific Programme "Young scientists and post-doctors" 2019/2020 together with Ministry of Education and Science of Bulgaria.

The Erufosine analytical standard was kindly provided by Prof. Spiro Konstantinov, DSci, (Medical University of Sofia, Faculty of Pharmacy, Department of Pharmacology, Pharmacotherapy and Toxicology, Sofia, Bulgaria).

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