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DETERMINATION OF DIHYDROERGOTAMINE *VIA* LC METHOD COUPLED WITH FLUORESCENCE DETECTION IN PHARMACEUTICAL FILM PREPARATIONS

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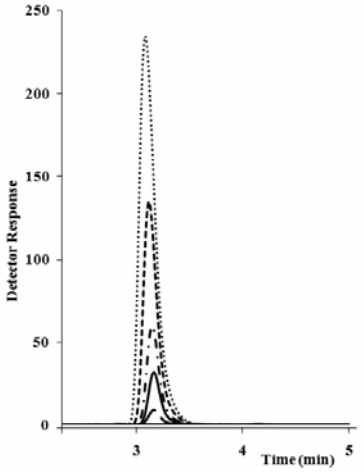
In this study, Dihydroergotamine Maleate was determined by LC coupled with fluorescence detector, using Agilent Eclipse XDB-C8 5 µm 4.6 x 150 mm column with a flow rate of 1.2 mL/min, at 25°C with mobile phase composition of 70:30 Buffer (containing 1.5% Formic Acid):ACN at an excitation wavelength of 280 nm and an emission wavelength of 350 nm. The analysis of Dihydroergotamine mesylate was obtained within 5 min. Sensitive determination method for the dihydroergotamine mesylate with LC coupled with fluorescence detector was achieved in range of 8.56 $x10^{-7}$ to $1.71x10^{-4}$ M with LOD value of $4.48x10^{-8}$ M and LOQ value of $1.36x10^{-7}$ M. The proposed method has been extensively validated in terms of precision, accuracy. Linear range, limit of detection and quantification values, are also calculated and discussed according to ICH Guidelines and USP criteria. The method is also used for the determination Dihydroergotamine in pharmaceutical film preparations and Dihydroergotamine was obtained from film formulations with an acceptable recovery results.



INTRODUCTION

Migraine is a disorder affecting about 10% of adults worldwide. It is a clinical syndrome of periodic moderate-to-severe headaches coupled with light sensitivity, nausea, and vomiting. Life quality of migraine patients reduces due to disability during attacks. Despite available treatments, social and leisure activities of patients decrease linked to migraine. Migraine pathophysiology is very complex and not completely understood also therapeutic advances have been slowly developing.[1-3]

Managing migraine is complicated for many reasons, including classification of headache syndrome, lack of biological markers, variable response to therapies and unknown pathophysiology. With these shortcomings, a systemic treatment is essential. Attention should be placed on the frequency of migraine attacks, associated symptoms, disability, and co-morbid conditions. Also tolerance of drugs may be taken into consideration due to patient's prior medication trials.[4-6]

Pharmacotherapy of migraine is divided into acute, or abortive, treatment aimed at symptom suppression and prophylactic treatment in which

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the goal is to reduce episode frequency, duration, and intensity.[4] Triptans are widely accepted as effective first-line therapy for moderate to severe acute migraine; however, some patients fail to achieve adequate pain relief and require additional treatment to alleviate migraine pain.[7-9] Lack of consistent response to a single triptan does not essentially predict non-response to other triptans or triptan formulations, some patients will not experience relief after numerous triptan trials.[10, 11]

Ergotamine and dihydroergotamine are non-triptan options for treating acute migraine. Dihydroergotamine mesylate (DHE), is an ergotamine derive used to stop or treat symptoms of an emerging migraine. DHE is formed by chemical reduction of an unsaturated bond on ergotamine, which results in anti-migraine efficacy similar to that experienced with ergotamine with substantially reduced vasocontractile and emetic effects. It acts as an agonist to the serotonin 5-HT(1D) receptors and causing vasoconstriction of the intracranial blood vessels. It also interacts centrally with dopamine and adrenergic receptors. It can be used to treat acute intractable headache or withdrawal from analgesics.[12-15]

Generally, DHE is given via intravenous (IV) injection or self-administered by intranasal delivery, intramuscular/subcutaneous injection or inhalation. Oral DHE is rarely used, owing to oral bioavailability of the parent compound is less than 1%, as a consequence of poor gastrointestinal absorption and substantial (>90%) first-pass metabolism of what little drug is absorbed. Intranasal administration also has low bioavailability, resulting in unexpected pharmacokinetic (PK) properties that may result a low clinical response. Given via the intravenous or intramuscular route, the absolute bioavailability of DHE is 100%; however, patient acceptance of intravenous DHE has been limited due to inconvenience of administration and side effects, especially nausea. At recommended dosages, by any route of administration, DHE has not been associated with serious cardiovascular adverse events. However, because of its known potential for arterial constriction and uterine contraction, DHE is contraindicated in some patients and the dose has to be set carefully.[1,13,15] In acute migraine, the speed of onset and sustained efficacy of the drug is desired. Also simple administered and well tolerated drugs could offer the convenience and therapeutic efficacy desired by acute migraine sufferers. In other words, patients are willing to trade inconvenience of administration for rapid and effective relief. [15-18]

In recent years, various drug delivery methods have been developed to deliver therapeutic agents through various routes to enhance the drug bioavailability and safety. As mentioned before, conventional dosage forms have many drawbacks and in this manner they are poor bioavailability and adverse effects due to high doses. These drawbacks can be overcome using fast dissolving sublingual films. This delivery system provides the maximum therapeutic efficacy and increases the bioavailability of drugs with minimum side effects and maximum stability and will also enable the drug to avoid the first pass metabolism, which is a major issue.[19,20] However, like intranasal administration, drug bioavailability is not expected to be 100% due to permeation from buccal mucosa. Taking into consideration of adverse effects the given dose must be arranged and determined carefully. Also excipient used in film formulations like propylene glycol can affect the determination.

In the literature there is some research based on dihydroergotamine and its analog determination such as determination of dihydroergocristine and dihydroergotamine using chromatographic method,[21] dihydroergotamine in plasma and urine.[22,23] In the light of this information, using these methods and modifying them, in this study a validated determination of DHE in film formulations was proposed via LC coupled with fluorescence detector.

EXPERIMENTAL

Chemicals and reagents

For the film preparations, DHE was obtained from USP (USA), Pullulan was procured from (China) and maltodextrin was obtained from Aldrich (Germany). Plasticizer, propylene glycol was obtained from Tekkim (Turkey). All the other chemicals used were of analytical grade and HPLC grade. Sodium hydroxide (Merck, Darmstadt, Germany) and o-phosphoric acid (Riedel-de Haen, Germany) was used for pH adjustment.

Film Preparations

The sublingual film of DHE was prepared by the solvent casting method (6). Aqueous solution was prepared by dissolving the maltodextrin and pullulan in 10 mL of distilled water, and stirred to produce a clear solution, then; plasticizer propylene glycol was added to distilled water and stirred for 1 h. The solution was kept at room temperature for 12 h to remove all air bubbles. The solution was cast in a petri dish of 9 cm in diameter, and was dried in oven at 60°C for 4 h. The film was then carefully removed from the petri dish and checked for any imperfection, and cut according to the size required for testing.

Preparation of Solutions

1000 $\mu g.mL^{-1}$ stock solution of DHE was prepared in methanol and diluted with mobile phase to desired concentrations.

The mobile phase was prepared by mixing solution containing 1.5% formic acid with acetonitrile in ratio of 70:30. For the mobile phase solutions, doubly distilled water with conductivity lower than 0.05 $\mu S.cm^{-1}$ was used and the mobile phase was filtered through 0.45 µm PTFE membranes using with a vacuum pump and degassed before the chromatographic run. The dead time (t_o) was measured by injecting KBr solution 0.01% (v/w), in water. All the solutions stored at +4 °C, kept away from light and used within 24 h to avoid decomposition.

Instrumentation for RP-LC

LC system consisting of a Hewlett-Packard (Avondale, USA) Model 1100 series with a Model Agilent series G-13159 UV detector and Model Agilent 1100 series G-1329 ALS auto sampler and HP chemstation was used. Chromatographic conditions were utilized using Agilent Eclipse XDB-C8 5 µm 4.6 x 150 mm column with a flow rate of 1.2 mL/min, at 25°C with mobile phase composition of 70:30 Buffer (containing 1.5% Formic Acid):ACN at an excitation wavelength of 280 nm and an emission wavelength of 350 nm.

System suitability test studies and Validation of the method

System suitability parameters including capacity factor symmetry (from integrator), symmetry (at 10% height), and tailing factor, theoretical number of plates were calculated related with USP criteria's.[24-26] Moreover, validation of the proposed method was also conducted including, precision, accuracy, and linear range, limit of detection and quantification values and discussed according to ICH Guidelines and USP criteria. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated from the equations of LOD= 3.3 s/m and LOQ = 10 s/m where "s" is the standard deviation of response) and "m" is the slope of the calibration curve.[27-29]

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

The optimum chromatographic condition was determined using different kinds of columns such as XSelect C18; 250 x 4,6; 5 µM, Eclipse XDB C18; 150 x 4,6; 5 µM, Thermo Hypersil Gold, C18; 250 x 4,6; 5 µM, XTerra C18; 250 x 4,6; 5 µM, different flow rates between 0.5-1.50 $mL.min^{-1}$, different percentages of different kinds of solvents such as methanol, acetonitrile, water, different buffering agents such as phosphoric aid, trifluoroacetic acid, and triethylamine. Finally, using Agilent Eclipse XDB-C8 5 µm 4.6 x 150 mm column with a flow rate of 1.2 mL/min, at 25°C with mobile phase composition of 70:30 Buffer (containing 1.5% Formic Acid):ACN at an excitation wavelength of 280 nm and an emission wavelength of 350 nm, the analysis of Dihydroergotamine mesylate was obtained within 5 min.

Validation and system suitability tests

In drug analysis, validation step is the most crucial step that should be reported in terms of precision, accuracy, and linear range, limit of detection and quantification values. Before the validation, system suitability tests should be performed. For the purposed method, under optimized conditions, to prove the suitability of the developed method, according USP 24, method <621>, system suitability test parameters were calculated and summarized in Table 1.

For the validation of the purposed method, precision of the method was checked by injecting 1 $\mu g.mL^{-1}$ DHE for successive 5 measurements, and the relative standard deviation (RSD %) values were calculated and found lower than 2. The RSD % values were given in terms of within day repeatability and between day repeatability, showing the precision results.

Table 1

System Suitability Tests Parameters

Parameters	DHE	Recommended Value[30]
Capacity Factor	2.16	>2
Selectivity to previous peak	5.76	>1
Selectivity to next peak	-	>1
Resolution to previous peak	5.28	>2
Resolution to next peak	-	>2
Tailing Factor	1.39	<2
Theoretical number of plates	3701	>2000

Increasing concentrations of DHE was injected to the system, and DHE was analyzed through linear regression analysis method. The DHE peak area *vs* DHE concentration plot was drawn from this plot, working range, LOD and LOQ values were calculated. Moreover, this plot was used to determine DHE in film preparations. In range of 8.56×10^{-7} to 1.71×10^{-4} M DHE, calibration graph was obtained with the calibration equation of y=49.99 x+19.52 with r value of 0.999, where y represents detector response and x is the concentration of DHE (figure 1). From this calibration graph, LOD and LOQ values were calculated with the LOD=3.3s/m and LOQ=10s/m relations, and sensitive determination method for the dihydroergotamine mesylate with LC coupled with fluorescence detector was achieved with LOD value of 4.48×10^{-8} M and LOQ value of 1.36×10^{-7} M. (Table 2).

Table 2

Statistical evaluation of the calibration data by RP-LC

Retention time (min)	3.16
Linearity range (M)	8.56×10^{-7} - 1.71×10^{-4}
Slope (mAU.M)	49.99
Intercept (mAU)	19.52
Correlation coefficient	0.999
LOD (M)	4.48×10^{-8}
LOQ (M)	1.36×10^{-7}
Within day Repeatability [a] (RSD %)	0.23
Between day Repeatability [a] (RSD %)	0.28

[a] Each value is the mean of five experiment.

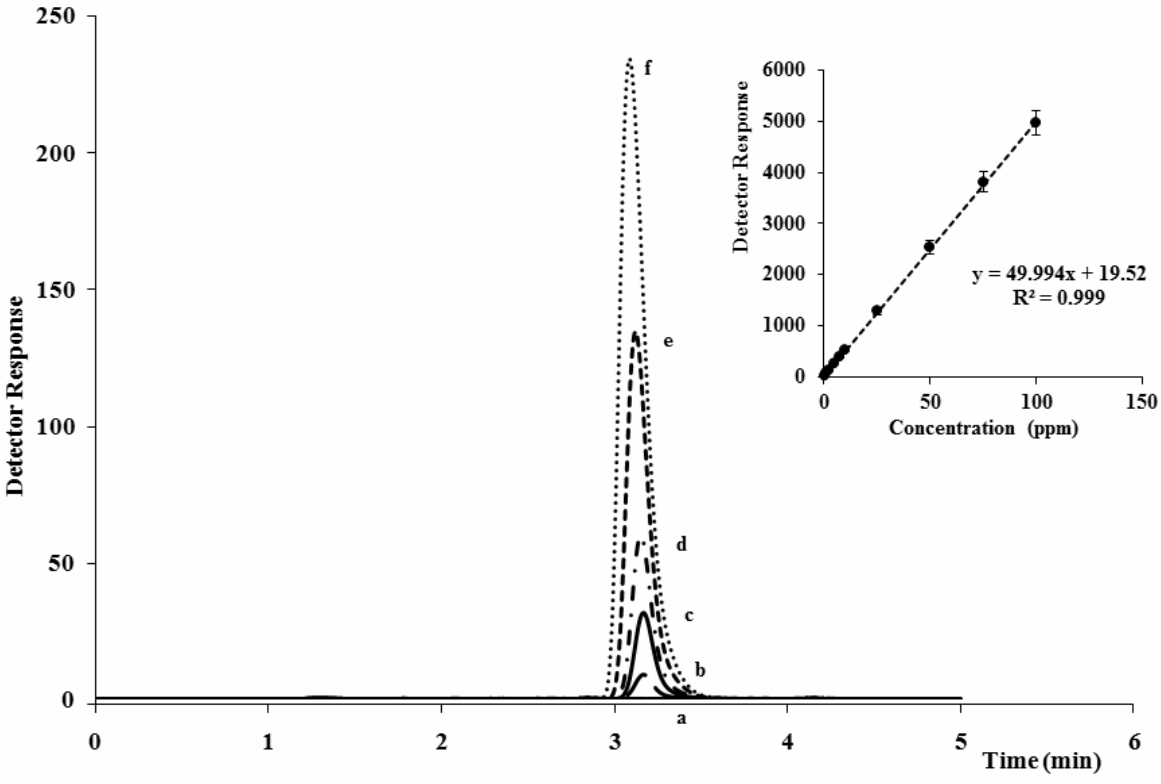

Fig. 1 – a) Chromatograms of increasing concentrations of DHE a) Mobile Phase b) 1µg.mL^{-1} c) 5µg.mL^{-1} d) 10µg.mL^{-1} e) 25µg.mL^{-1} f) 50 µg.mL^{-1} Inset. Calibration plot detector response vs concentration of DHE.

Analysis of dihydroergotamine from film preparations

SEM images related with the designed film were shared in figure 2. From SEM images it is clear that soft, plain films were obtained. Films were containing 10 μg.mL^{-1} DHE, were dissolved in methanol and analyzed with the purposed method. Using this method, each film was found containing 9.89 μg.mL^{-1} DHE (figure 3). Moreover, recovery studies were performed, on the film preparations by adding known amount of DHE (5 μg.mL^{-1}) to film preparations containing 10 μg.mL^{-1} DHE. The results were shared in Table 2.

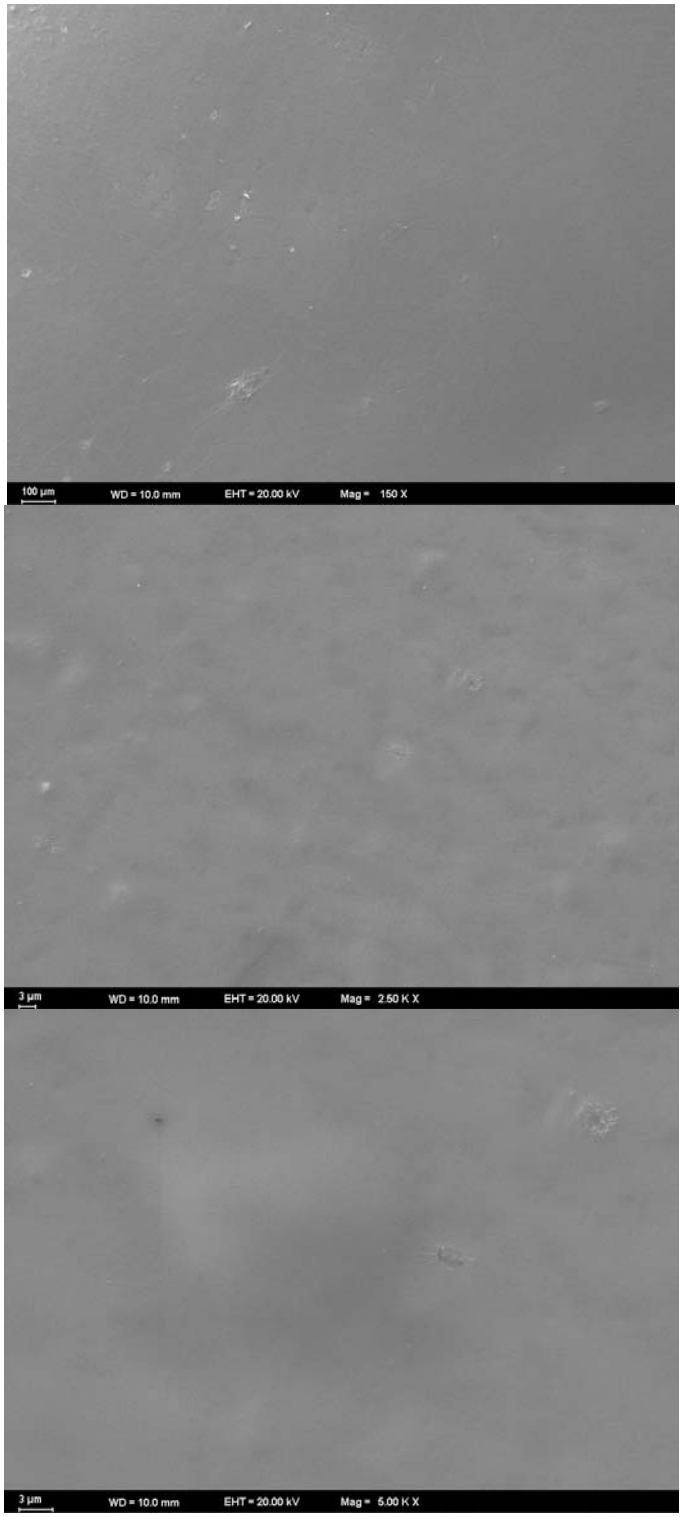

Fig. 2 – SEM images of film preparations with different magnitudes.

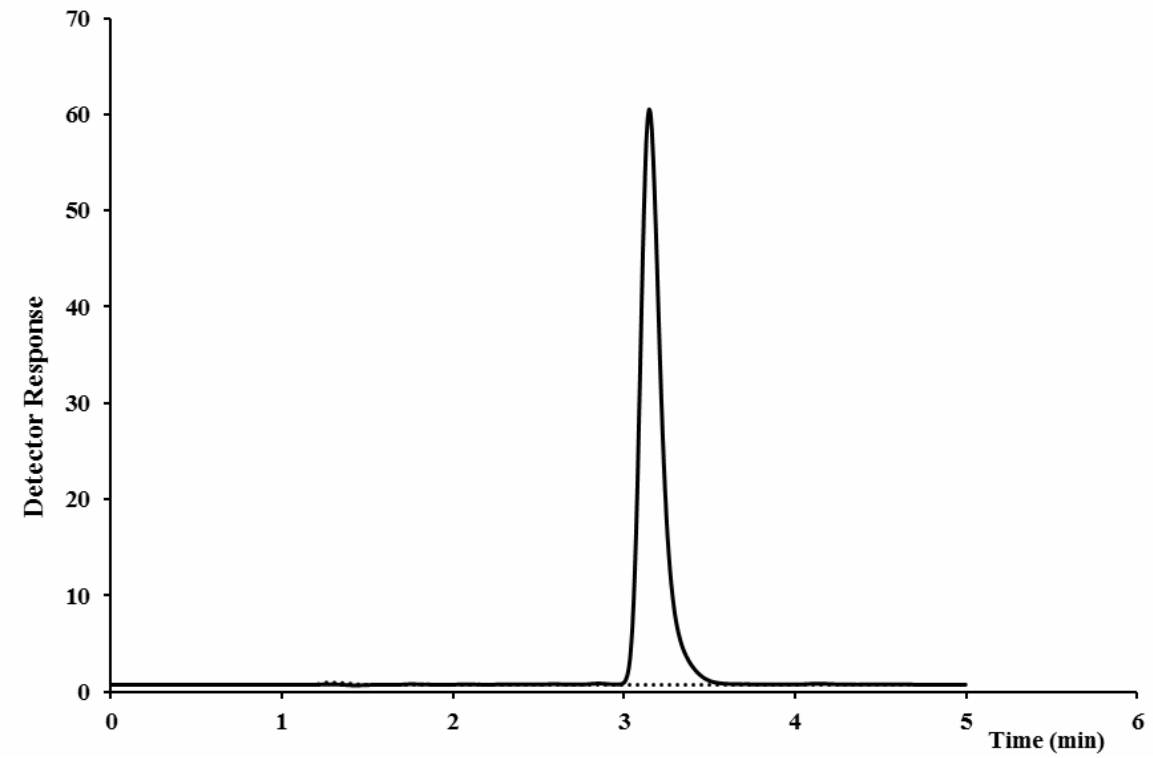

Fig. 3 – Chromatograms of DHE from film formulations.

Table 3

Results from film preparations and recovery analysis

Film preparation contains (μg.mL^{-1})	10.00
Found[a] (μg.mL^{-1})	9.89
RSD (%)	0.21
Bias (%)	1.69
Added (μg.mL^{-1})	5.00
Found[a] (μg.mL^{-1})	5.09
Recovery (%)	101.8
RSD of recovery (%)	0.23
Bias (%)	-1.8

[a] Each value is the mean of five experiment.

CONCLUSIONS

In this study a validated determination of Dihydroergotamine mesylate, which is an ergotamine derive used to stop or treat symptoms of an emerging migraine, in film formulations was proposed via LC coupled with fluorescence detector using Agilent Eclipse XDB-C8 5 μm 4.6 x 150 mm column with a flow rate of 1.2 mL/min, at 25°C with mobile phase composition of 70:30 Buffer (containing 1.5% Formic Acid):ACN at an excitation wavelength of 280 nm and an emission wavelength of 350 nm. With this purposed method, the analysis of Dihydroergotamine mesylate was obtained within 5 min with the LOD and LOQ values of 4.48x10^{-8} M and LOQ value of 1.36x10^{-7} M, respectively. Moreover, the proposed method has been extensively validated in terms of precision, accuracy, linear range, limit of detection and quantification values, system suitability tests were also performed according to ICH Guidelines and USP criteria.

The various drug delivery methods have gained great importance as drug delivery techniques can enhance the drug bioavailability and safety, the maximum therapeutic efficacy and can increase the bioavailability of drugs with minimum side effects and maximum stability and can also enable the drug to avoid the first pass metabolism. The developed LC method is also used for the determination

Dihydroergotamine in pharmaceutical film preparations and Dihydroergotamine was obtained from film formulations with an acceptable recovery results.

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