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Lumefantrine: A Review on Analytical Methods

D. Mounika[1]*, R. Shanmugam[1], A. Mohana Krishna[1], A. Kirthi[1], M. Shanthi prathyusha[1], G Shoba Rani[1]

1. Department of Pharmaceutical Analysis, Sree Vidhyanikethan College of Pharmacy, Sree Sainath Nagar, Tirupati-517 102, Andhra Pradesh, India.

ABSTRACT

Lumefantrine is a antimalarial drug which is widely used in malaria endemic areas. For anti-malaria therapy Lumefantrine is used in combination with Artemether under the brand name of co-artem. Lumefantrine have an endoperoxide bridge, which interferes with haeme polymerization critical detoxifying pathway for the malaria parasite and also inhibiting in the nucleic acid and protein synthesis within the parasite as a secondary action. The Lumefantrine-Artemether combination is the first line therapy recommended by Brazilian Health Ministry to the falciparum malaria treatment (Brasil, 2006) now a days. This article examines published analytical methods reported so far in the literature for the determination of Lumefantrine in biological samples and pharmaceutical formulations; they include various techniques like spectrophotometer, high pressure liquid chromatography, liquid chromatography-electrospray ionization tandem mass spectrometry and high-performance thin layer chromatography.

Keywords: Lumefantrine, Analytical methods, Anti malarial.

*Corresponding Author Email: mounidamacharla@gmail.com
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INTRODUCTION

Chemically Lumefantrine is a (2-Dibutylamino-1-[2, 7-dichloro-9-(4-chlorobenzylidene)-9Hfluoren-4-yl] ethanol, with a molecular formula $C_{30}H_{32}Cl_3NO$ and having molecular weight of 528.9 g mol-1. Lumefantrine is a yellow crystalline powder which is practically insoluble in water and aqueous acid. Lumefantrine act as blood schizontocides. Lumefantrine has the character to interfere with the haem polymerization process, a critical detoxicifying pathway for the malaria parasite. Lumefantrine also have a Secondary action involving inhibition of nucleic acid and protein synthesis within the malarial parasite[1]. Lumefantrine (benflumetol) is a 2, 4, 7, 9-substituted fluorine (2, 3-benzindene) derivative (Figure 1). It was synthesized in the 1970s by the Academy of Military Medical Sciences, in Beijing, and registered in China for anti-malarial use in 1987. Lumefantrine is now commercially available in fixed combination products, mostly with β-Artemether (ACT, Artemisinin-based combination therapy). Lumefantrine-Artemether combination proven to be highly efficacious for treatment of uncomplicated falciparum malaria[2]. In the present review we have compiled the published analytical methods reported so far in the literature for determination of Lumefantrine in biological samples and pharmaceutical formulations with techniques like Potentiometry, spectrophotometry, high-performance liquid chromatography (HPLC), liquid chromatography- mass spectrometry (LC-MS) and high-performance thin layer chromatography (HPTLC) have been used for analysis.

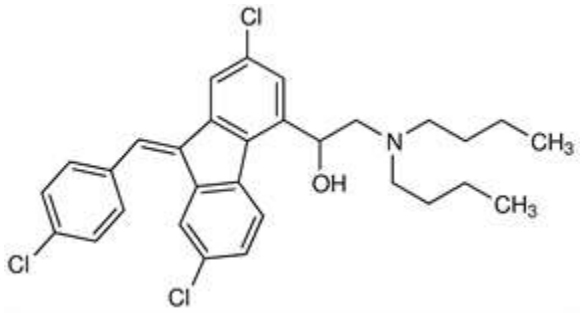

Figure 1: Chemical structure of lumefantrine

Physicochemical characters

Lumefantrine is a fine and having the characteristics like yellow crystalline powder, odorless, practically soluble in water, freely soluble in ethyl acetate, soluble in dichloromethane, slightly soluble in ethanol, chloroform and acetonitrile[3-5].

Biopharmaceutics classification System (BCS)

Bio pharmaceutical classification for the Lumefantrine falls under BCS-class IV drugs, which shows the drug was poorly soluble and poorly permeable. In order to enhance the solubility and dissolution rate of the drug various strategies like solid dispersion and nanotechnology approach were carried[6-10].

Analytical Methods

Spectrophotometry

In the literature few methods were reported for the estimation of Lumefantrine using spectrophotometry[12-14], of which some methods are for determining Lumefantrine alone, whereas the remaining are for quantifying Lumefantrine in combination with other drug substances. Table 1 shows the summary of the reported spectroscopic methods indicating the basic principle, $\lambda_{max,}$ solvent and limit of detection (LOD).

Table 1: Representative spectrophotometric methods for analysis of Lumefantrine

Compounds	Method	λ_{max}	Solvent	LOD (µg/ml)	Ref.
Lumefantrine	Spectrophotometric method	228	Methanol	0.7	12
Lumefantrine	Simultaneous equation method	252	0.01N NAOH	0.7	13
	Absorbance ratio method	268		0.4	
	Dual wave length method	296		0.2	
Lumefantrine	Spectrophotometric method	234	Methanol	0.04	14

Electrochemical methods

The Lumefantrine is also determined by potentiometric titration methods to quantify lumefantrine in raw materials and tablets[15]. Non-aqueous titration of Lumefantrine was carried out using Perchloric acid as titrant and glacial acetic acid/acetic anhydride as solvent. The end point was potentiometrically determined.

Chromatography

HPLC

Biological samples

Developed the method for determination of Lumefantrine in Human Plasma by HPLC-UV. Lumefantrine and its internal standard halofantrine were extracted from plasma samples using protein precipitation with acetonitrile (0.2% Perchloric acid)followed by solid-phase extraction with Hypersep Cs cartridges. Chromatographic separation was performed on a zorbax SB-CN HPLC column (3.0×150mm, 3.5µm) with water/methanol (0.1%TFA) as the mobile phase in a gradient elution mode. Detection was performed using UV/vis detector at 335nm wave length[16]. The method showed to be linear over a range of 50-10,000 ng/ml with acceptable intra and inter-day precision and accuracy[17]. A field adapted sampling and HPLC quantification method for determination of Lumefantrine and its desbutyl metabolite in whole blood spotted on filter paper. The samples were separated on a Zorbax Eclipse XDB –phenyl column (4.6 mm x 150 mm, particle size 5µm, flow rate of 1ml/min) using a mixture of acetonitrile-0.1 ammonium acetate buffer, 0.01 M acetic acid, pH 6.5 (10:90v/v) .detection was achieved at 335nm[18]. A simple, high throughput HPLC with UV detection method was developed for the determination of Lumefantrine and its metabolite in plasma. The separation was carried out using Synergi C18 column 250x3mm,5µm using a mixture of acetonitrile:

ammonium acetate buffer, pH 4.9 (85:15v/v) with flow rate 1.5ml/min by the detection wave length335nm.

Pharmaceutical samples

Analytical methods for the determination of Lumefantrine in pharmaceutical dosage forms using HPLC are shown in the table 2.

Table 2: Reported analytical HPLC methods for determination of Lumefantrine combination with Artemether in pharmaceutical dosage form

Study aim	Mobile phase	Column	Detection	λ_{max} (nm)	Flow rate (ml/min)	LOD (µg/ml)	Ref.
Stress degradation studies on Lumefantrine	Water (pH adjusted to 3 with orthophosphoric acid): acetonitrile: methanol in the ratio of 4:5:1. (*v/v*)	RP Spherisorb C-18 (Waters) column with dimensions (250 mm × 4.6 mm, 5-µm particle size)	PDA detector	266	1	0.05	19
Simultaneous estimation of Artemether and Lumefantrine	potassium dihydrogen orthophosphate buffer and acetonitrile in the ratio of 40:60(v/v), pH 3 ± 0.5	symmetry C18, 250 x 4.6 mm , 5-µm particle size	UV	210, 303	1.5	-	20
Simultaneous estimation of β-Artemether and Lumefantrine	Phosphate buffer and acetonitrile 52:48 (v/v) ,pH- 3.0	Three reversed- phase fused- core HPLC columns (Halo RP-Amide, Halo C18 and Halo Phenyl-hexyl)	UV	210, 335	1	0.1	21
Simultaneous determination of Artemether and Lumefantrine	methanol: 0.05 % trifluroacetic acid with triethylamine buffer pH 2.8 adjusted with orthophosphoric acid in ratio (80:20 v/v)	Hypersil ODS C18 (250mm□ ~4.6mm□ ~5µparticle Size)	PDA detector	210	1.5	0.0004	22
Simultaneous estimation of Artemether and Lumefantrine	Acetonitrile and 0.01M potassium dihydrogen orthophosphate (70:30) ,PH -4	Hypersil (BDS) C18 (250× 4.6mm, 5µm)	UV	254	1	0.01	23
Simultaneous estimation of Artemether and Lumefantrine	Acetonitrile; buffer (0.1% ortho phosphoric acid,pH 3) 60:40(v/v)	Symmetry C 18 (250x 4.6mm, 5µ)	UV	303	1.5	-	24
Determination of Lumefantrine in pharmaceutical product	Methanol and 0.05%trifluoroacetic acid (80:20)	Symmetry C18 (250x 4.6mm, 5µ)	UV	335	1	0.02	25
Determination	Water:acetonitrile;glacial	Symmetry C18 (250x	UV	276	1.2	-	26

of Lumefantrine in pharmaceutical dosage forms	acetic acid (48:52:1)v/v/v	4.5mm, 5µ)					
Estimation of Lumefantrine in solid dosage forms	Acetonitrile and methanol (50:50)% v/v	Symmetry C18 (250x 4.5mm, 5µ)	UV	235	2	-	27

LC-MS

The quantification of Lumefantrine in human plasma using LC-MS/MS and its application to Bioequivalence studies[28]. This method was developed based on protein precipitation and chromatographic separation was carried out on a shimadzu LC with a Inertsil ODS-2V column (50× 4.6 mm, 5µm) using methanol, acetonitrile and 0.1% formic acid in water solution in the ratio of 56:24:20 v/v/v at a flow rate of 1ml/min. electrospray ionization was chosen for this method. The pharmacokinetic parameters evaluated were $C_{max,\ AUC0\text{-}7}$, T_{max}, kel and $T_{1/2}$. The method provided excellent specificity and linearity with limit of quantification of 200 ng/ml for Lumefantrine. For the determination of Lumefantrine and its metabolite Desbutyl-Lumefantrine in plasma from patients infected with plasmodium falciparum malaria[29]. Samples were extracted with simple solvent precipitation procedure, the samples were loaded onto Oasis HLB 1cc (30mg) extraction columns. The separation was achieved using XTerra RP18 (2.1mm × 100mm, 5.0µm) column using 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) as a binary gradient solvent system. Mass detector was performed using a triple quadrupole mass spectrometer operating in positive electrospray ionization mode. The method was applied to the determination of the concentrations of LF and DLF in patients infected with plasmodium falciparum malaria on day 3 and 7 after treatment with lumether. For the determination of Lumefantrine in small-volume human plasma LC-MS/MS: using a deuterated Lumefantrine to overcome matrix effect and ionization saturation[30]. The system consisted of twin PE 200 auto sampler, and the API 2000 triple quadrupole MS system. Separation was carried by using Zorbax C8 column (50 × 2.1mm, 5µm) using aqueous ammonium formate 10M at pH 4.0 (solvent A) and acetonitrile with formic acid 0.1% (solvent B) as solvents. Deuterated Lumefantrine used as the internal standard.

HPLC-DAD/UV-ESI/MS

Developed a stability- indicating HPLC-DAD/UV-ESI/MS impurity profiling of the anti-malarial drug Lumefantrine[31]. Using this method, a comprehensive impurity profile was established upon analysis of market samples as well as stress, accelerated and long-term stability results. In-silico toxicological prediction for these Lumefantrine related impurities

were made using Toxtree and Derek. HPLC-UV investigation of the impurity profiles was performed on a HPLC-PDA apparatus consisting of a waters Alliance 2695 separation module and a waters 2998 photodiode array detector with empower 2 software for data acquisition. For PDA detection, quantification was performed at 266 nm. The positive ion ESI and the collision induced dissociation (CID) mass spectra were obtained from the LC-UV/MS apparatus consisting of a spectra system SN4000 interface, a spectra system SCM1000 degasser, a spectra system P1000XR pump, a spectra system AS3000 auto sampler, and a Finnegan LCQ classic ion trap mass spectrometer in positive ion mode.LC determination of impurities in lumefantrine samples was performed using a purospher STAR RP-18 end capped (150 × 4.6 mm, 5 μm particle size) using ammonium acetate(Ph 4.9; 0.1M) and acetonitrile(10:90, v/v) as mobile phase with flow rate 2ml/min.

CONCLUSION

There are a wide range of techniques are available for the analysis of Lumefantrine in pharmaceutical formulations and biological samples. The analysis of published data revealed that HPLC method was extensively used for the estimation of Lumefantrine in various matrices like urine, plasma and serum. HPLC-MS/MS is recommended for determination of Lumefantrine in biological, because this method combines the HPLC separation ability with MS sensitivity and selectivity which allows the unambiguous identification of Lumefantrine and its metabolites. HPLC with UV detection is applicable in case of analysis of Lumefantrine in pharmaceuticals; this article provides the information to reviewers and researchers about determination of Lumefantrine by various analytical methods.

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