

SOLID PHASE EXTRACTION (SPE) APPLICATION

Automated 96-well SPE and LC-MS/MS using ESI for simultaneous determination of Artemether and its active metabolite Dihydroartemisinin in human plasma

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Introduction:

Malaria is a leading cause of mortality and morbidity in the developing world, and resistance to available drugs is increasing. A synthetic trioxolane antimalarial drug discovered only 30 years ago Artemether, is an active synthetic derivative of Artemisinin a traditional herb used in China for over 2000 years against fevers. To date, pharmacokinetic studies on plasma levels of Artemether and its active metabolite Dihydroartemisinin have been accomplished with liquid-liquid extraction¹⁻⁴ followed by APCI-SIM⁵. We present here a process critical to sensitive and reproducible PK studies made possible with simultaneous detection of both the parent ion and its metabolite.

Novel Aspect:

PK studies in 96-well format with EDTA-plasma were accomplished for Artemether, and its metabolite Dihydroartemisinin via LC-MS/MS of SPE eluates.

Abstract:

A rapid and sensitive method for the determination of Artemether and its active metabolite DHA has been developed on automated solid phase extraction (SPE) and high performance liquid chromatography with electrospray tandem mass spectrometry (LC-MS/MS). The SPE method was automated on a 96-well extraction plate (Orochem Celerity Deluxe DVB LP 30mg 1cc) with 96 channel programmable liquid-handling workstation, and coupled off-line with 96-head positive pressure processor. The chromatographic separation was developed using a simple isocratic mixture of 2mM Ammonium acetate Buffer: Acetonitrile (20:80), followed by sample introduction through an ionspray interface in the positive ion mode and tandem mass spectrometry detection with multiple reaction monitoring. Method for SPE was optimized by using different EDTA plasma from different sources. The plasma was subjected to SPE plate to remove protein and other interfering impurities in the plasma to get clean extract for LC-MS/MS analysis. Sample clean up using 96 well polymer DVB LP 30mg 1cc reduces the background noise produced by ESI, enabling the development of a single, and quicker method for the simultaneous extraction of Artemether and its active metabolite Dihydroartemisinin, DHA. Compared to the conventional solid phase extraction using vacuum manifold, the automated high throughput SPE method using positive pressure processor, afforded significant time savings in sample preparation. The assay method was validated and applied successfully to the analysis of Artemether, and DHA in pharmacokinetic and bio-equivalence studies.

Methods:

A manual SPE method of extraction established using 20-head vacuum manifold was transferred to the Orochem Celerity Deluxe polymer SPE cartridges in 96-well format using the Oroflex Personal Pipettor, an Orochem Technologies Inc. robotic liquid handling system. Pre-conditioning and equilibration, was accomplished using the Oroflex robotic 96 channel Pipettor and off-line processing with the EZYPRESS

an Orochem positive pressure processor. The eluate was collected through positive pressure processing in a 96 -well format and directly injected into an HPLC system using 96 well autosampler. Detection of the parent ion and the metabolite was accomplished by mass spectrometry using multiple reaction monitoring mode. Results are presented for LLOQ, LQC, MQC and HQC (N=6), with 3-day inter-assay measured for precision and accuracy.

Results:

Both the drug and metabolite were validated successfully. The resulting assay was sensitive, selective, precise and accurate. Inter assay Precision and accuracy ranged from 4.6 to 9.7 % and 95.7 to 106.4 %

Batch No.	Nominal Concentration (ng/ml)							
	2.5	5	20	40	80	100	150	200
P&A – I	2.6	4.5	20.3	38.5	81.4	90.2	156	202
P&A – II	2.6	4.6	19	41.8	79.5	95.3	167	218
P&A – III	2.5	5.2	18.3	42.6	78.2	87.5	165	207
N	3	3	3	3	3	3	3	3
Mean	2.6	4.8	19.2	41.0	79.7	91.0	162.7	209.0
SD (±)	0.090	0.390	1.015	2.173	2.263	5.515	6.364	8.185
Precision (%CV)	3.5	8.2	5.3	5.3	2.8	6	4	3.9
Nominal (%)	102.5	95.4	96	102.4	99.8	91.4	107	104.5

Table 1: Back Calculated Calibration Standard Statistics for Artemether

Batch No.	Nominal Concentration (ng/ml)							
	2.5	5	20	40	80	100	150	200
P&A – I	2.59	4.6	20.2	39.2	80.9	95.1	153	203
P&A – II	2.62	4.5	20.5	39.5	75.6	98.8	162	205
P&A – III	2.51	4.9	19.9	38.3	82.3	95.9	155	205
N	3	3	3	3	3	3	3	3
Mean	2.57	4.71	20.2	39	79.6	97.4	156.7	204.3
SD (±)	0.057	0.251	0.3	0.624	3.534	2.051	4.726	1.155
Precision (%CV)	2.2	5.3	1.5	1.6	4.4	2.1	3	0.6
Nominal (%)	102.9	94.1	101	97.5	99.5	97.4	104.4	102.2

Table 2: Back Calculated Calibration Standard Statistics for Dihydroartemisinin (DHA)

Nominal Concentration	2.5	7.5	50	120
Average Concentration	2.48	7.6	52.0	127.7
Standard Deviation	0.239	0.557	2.376	8.830
Precision (%)	9.7	7.3	4.6	6.9
Accuracy (%)	99.1	101.2	104.0	106.4
N	18	18	18	18
Batch No.				
P&A I	2.7	7.0	51.5	131
	2.8	8.3	51.9	129
	2.3	8.6	55.6	120
	2.3	6.6	50.5	137
	2.3	7.8	57.0	130
P&A II	2.6	8.1	53.5	127
	2.3	7.0	54.3	151
	2.2	7.1	51.9	127
	2.3	8.2	50.1	120
	2.1	7.7	53.8	124
P&A III	2.6	7.7	50.5	136
	2.8	7.9	51.1	132
	2.3	7.4	52.1	124
	2.3	7.6	50.3	121
	2.8	7.9	53.4	122
2.7	7.4	52.6	134	
2.7	7.6	47.9	110	

Table 4: Inter-Assay Quality Control Sample for DHA

Nominal Concentration	2.5	7.5	50	120
Average Concentration	2.54	7.4	48.2	114.8
Standard Deviation	0.171	0.623	3.366	8.002
Precision (%)	6.7	8.5	7.0	7.0
Accuracy (%)	101.8	98.3	96.5	95.7
N	18	18	18	18
Batch No.				
P&A I	2.5	7.4	50.8	128
	2.3	7.5	51.9	114
	2.7	8.3	51.7	109
	2.2	6.7	54.0	129
	2.5	6.5	50.5	116
P&A II	2.9	7.2	45.2	110
	2.3	6.8	44.0	109
	2.6	6.9	43.9	112
	2.4	8.4	45.0	104
	2.8	6.6	44.5	106
P&A III	2.5	7.5	46.0	109
	2.6	6.8	47.0	112
	2.4	8.1	51.8	128
	2.6	7.9	47.7	116
	2.6	7.4	50.7	111
2.7	7.2	47.1	108	
2.7	6.9	47.5	117	
2.7	8.1	44.7	121	

Table 3: Inter-Assay Quality Control Sample