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Development and Application of Rp-Hplc Method for Dissolution Study of Oral Formulations Containing Amlodipine Besylate

Harshal Ashok Pawar* and Akanksha Yadav

Dr. L. H. Hiranandani College of Pharmacy, Ulhasnagar, Maharashtra, India

Abstract

In the present analytical study a rapid, robust and specific reversed-phase HPLC method has been developed and validated for quantitative estimation of amlodipine besylate in the dissolution study of oral films by direct injections of aqueous solutions. The study involved isocratic elution of amlodipine besylate in Zorbax® Eclipse XDB-C18 analytical column using buffer (0.7 % aqueous triethylamine adjusted to pH 3.0 with orthophosphoric acid) and methanol in the ratio of 40:60 (v/v). The aqueous solutions were analysed at a flow rate of 1.0 ml/min at 239 nm. The method presented linearity (r^2= 0.999) in the concentration range 20-150 µg/ml. The result indicated good recoveries ranging from 98.06% to 99.22%. The method showed good precision with % Relative standard deviation value less than 2. All the validation parameters were within the acceptance range. The developed method can be successfully employed for *in-vitro* dissolution and routine analysis of formulations containing Amlodipine besylate.

Keywords: Liquid chromatography; Amlodipine besylate; Dissolution; Elution; Method validation; Pharmaceutical film

Introduction

Amlodipine besylate (AMB) {3-Ethyl 5-methyl (4RS)-2-[(2-aminoethoxy) methyl]-4(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzenesulfonate} is a dihydropyridine calcium channel blocker indicated for the treatment of hypertension and Coronary Artery Disease (Figure 1)[1,2]. Its molecular formula is $C_{20}H_{25}ClN_2O_5{\cdot}C_6H_6O_3S$, formula weight is 567.05 [3]. Amlodipine is well absorbed following oral administration resulting in moderately high absolute bioavailability of 64% [4]. Following administration of a single 10mg oral dose, the mean time to peak concentration (T max) of 7.6 h and 6.4 h is reported and is 97% bound to plasma proteins [5]. It is extensively but slowly metabolised [6]. AMB is available in tablet and capsule dosage forms and is recommended as monotherapy or as a component of combination therapy for the treatment of angina pectoris and hypertension [7]. Some of the brands include Norvasc®, Caduet®, Lotrel®, Azor® and Tekamlo® [8]. AMB is available in 2.5, 5 and 10 mg oral dose alone and in combination with other drugs with 10 mg/day being the maximum daily dose. AMB orally disintegrating tablet is official in the United States Pharmacopoeia [9]. Literature survey revealed voltametric determination of AMB in human urine and pharmaceuticals [10]. Also, LC-MS and planar chromatography method was reported in biologicals [11]. Planar chromatography methods are also reported for bulk drug and combination tablet dosage form [12-14]. GC method was also proposed following trimethylacetyl derivatization in human plasma as matrix using ECD [15]. GC-MS, HPLC, spectroscopic methods are also reported for drug substance and AMB pharmaceutical formulation (tablets) [16-22]. Films for oral drug delivery most recently were made official in the European Pharmacopoeia, edition 7.4 and subordinated to the monograph 'oromucosal preparations' [23]. Since, the literature survey did not reveal determination of AMB in oral film formulation by HPLC, the present research work was undertaken (Figure 1).

Materials and Methods

Chemicals and reagents

Amlodipine besylate (99.85% purity) was obtained from Flamingo Pharmaceuticals Ltd., Mumbai, India. HPLC grade methanol and orthophosphoric acid were procured from Rankem, Mumbai, India. Triethylamine was obtained from SD Fine chemicals, Mumbai, India. All the chemicals and reagents used were of AR grade.

Instrumentation and chromatographic conditions

HPLC system (Agilent Technologies 1200 series) equipped with quaternary pump, manual sampler, column heater and UV-visible Variable Wavelength Detector (1200 VWD) was employed for analysis. Analysis of aqueous solutions was carried out at 239 nm with Zorbax® Eclipse XDB-C18 reversed–phase analytical column (4.6 x 150 mm, 5µm). The isocratic mobile phase consisted of a mixture of buffer (0.7 % aqueous triethylamine adjusted to pH 3.0 with orthophosphoric acid) and methanol in the ratio of 40:60 (v/v) throughout the analysis. The mobile phase was degassed by vacuum filtration before use. The flow rate of the mobile phase was 1.0 ml/min and total run time was 8 minutes. The column temperature was controlled at 30°C and

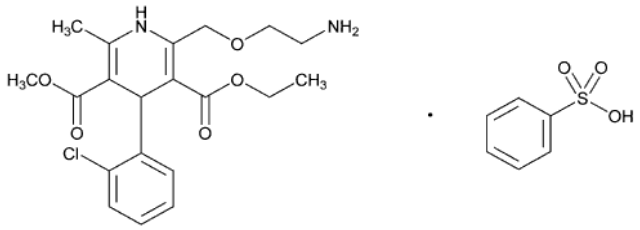

Figure 1: Structure of Amlodipine besylate.

***Corresponding author:** Harshal Ashok Pawar, Assistant Professor & Head of Department (Quality Assurance), Dr. L.H.Hiranandani College of Pharmacy, Smt. CHM Campus, Opp. Ulhasnagar Railway Station, Ulhasnagar-421003, Maharashtra, India, Tel: +91-8097148638; E-mail: hapkmk@rediffmail.com, harshal.dlhhcop@gmail.com

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injection volume was 20 µl. Chromatographic data was acquired using ChemStation software (Agilent Technologies).

Preparation of AMB standard solution

AMB stock solution was prepared by dissolving 10.0 mg of AMB in 0.01 M HCl by sonication into 100 ml volumetric flask and the final volume was made using 0.01 M HCl to get the final concentration of 100 µg/ml. Further dilutions were made to obtain standard solution of 5µg/ml.

Formulation development

Oral films of AMB 2.5 mg were prepared by solvent casting method using synthetic and natural polymers. The prepared polymer solution was casted on polypropylene petri dish, dried and the film formed was utilized for further evaluation and HPLC analysis [24,25]. The oral films were evaluated for drug content and the results obtained were within accepted limit.

Dissolution testing

USP XXIII paddle (USP type II) dissolution apparatus was used with 500 ml of 0.01 M HCl as dissolution medium that was freshly prepared each day of use and maintained at 37±0.5°C with 50 rpm for analysis [26]. Each dissolution was performed with six film samples. The aliquots of samples were withdrawn at the end of dissolution (at 270 seconds). These samples were centrifuged for 20 minutes and analysed by HPLC.

Method validation

The method was validated for parameters like precision, linearity, accuracy, specificity, robustness as per the ICH guideline [27-32].

System precision: Precision is the degree of variability in the results using the same method and is normally expressed as percent relative standard deviation (%RSD) and must be less than 2% for most methods. Six replicate injections of the standard solutions were injected for analysing system precision.

System suitability testing: System suitability tests are performed to verify that the chromatographic system is adequate for the intended analysis. Six replicate injections were injected before sample analysis. The acceptance criteria for system suitability testing were <2% RSD for peak area, USP tailing factor <2 and theoretical plates >2000. The results were used to monitor critical operational parameters to confirm precision of the chromatographic system prior to analysis.

Specificity: The specificity of the method was evaluated by injecting the placebo solution having same concentration as that of sample. The placebo were transferred to dissolution vessels of dissolution apparatus with same operating conditions as that for samples, centrifuged and analysed. All chromatograms were examined for any co-elution of the drug with excipients. The interference of the excipients of each formulation was examined.

Linearity: The linearity (range) of a method refers to the ability of a method to give accurate results over a given range (high and low) of analyte concentrations. Standard calibration curves were prepared at concentration levels from 20% to 150% of the standard concentration and the data was statistically confirmed with linear least square regression analysis.

Method Precision: Method precision (repeatability) is determined by multiple analyses of the same sample solution over a short period of time. Precision of the method was evaluated on six independent dissolution test samples of drug containing formulation.

Recovery: Recovery (accuracy) is the amount of the compound of interest analyzed as a percentage to the theoretical amount present in the medium. Accuracy was checked at three concentration levels 50%, 100% and 150% by standard addition method. The pre-analyzed placebo solutions were spiked with the determined drug concentration at three levels and further evaluated for recovery.

Robustness: Robustness of the method is the ability to withstand small changes in running conditions. Robustness of the method was determined by making the following deliberate changes in the chromatographic conditions: The flow rate of the mobile phase ±0.2 ml/min (0.8 ml/min, 1.2 ml/min) detection wavelength ±2 nm (237 nm, 241 nm). Standard solution was injected three times for each change.

Ruggedness: Ruggedness (Intermediate precision) of the method is the degree of reproducibility of the test results of the same sample under variety of normal conditions. Ruggedness of the method was evaluated by injecting six dissolution sample solutions using different analysts on different days.

Solution stability: Standard solutions and sample solutions (4, 5 and 6 µg/ml) were stored at room temperature for 24 hrs and analysed by HPLC by injecting at different time intervals until the study period against freshly prepared solutions.

Filter Evaluation: The filter evaluation is necessary to ensure that it does not adsorb drug and that it removes insoluble excipients that may otherwise cause high background or turbidity. Standard and sample solutions were prepared in the dissolution medium, the solutions were either filtered through Whatman No. 41 filter paper or centrifuged. In both the tests standard and sample solutions were analysed for its peak response factors.

Results and Discussion

λ_{max} for AMB was determined by UV-VIS spectroscopy by preparing standard solution in the dissolution media (0.01 M HCl). The peak of maximum absorbance wavelength (max) was observed. The result indicated λ_{max} at 239 nm with 0.3021 AU which was further used for HPLC method development. The UV spectrum for AMB is indicated in the figure 2.

HPLC method development

Amlodipine besylate is less polar molecule hence strongly retained on reversed-phase HPLC columns and is freely soluble in organic

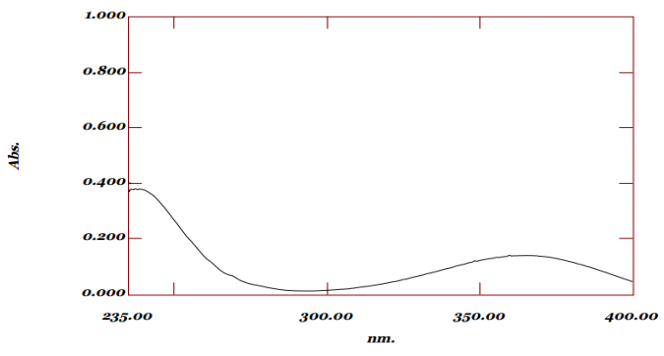

Figure 2: UV spectrum of AMB.

solvents like methanol, ethanol, DMSO. The column selection has been done on the basis of backpressure, peak shape, theoretical plates and day-to-day reproducibility of the retention time on Hypersil® BDS C18 column and Zorbax® Eclipse XDB-C18 column.

During method development, use of acetonitrile and methanol resulted in asymmetric peaks and peak tailing >2. To reduce the run time and improve the peak symmetry, the concentration of the organic portion of the mobile phase was varied. Although, USP peak tailing was observed >2. Hence, triethylamine (0.7% aqueous solution) was added to minimise peak tailing. At the reported concentration of buffer (0.7 % aqueous triethylamine adjusted to pH 3.0 with orthophosphoric acid) and methanol in the ratio of 40:60 (v/v) the USP tailing factor was within the acceptable limit resulting in good peak symmetry. A flow rate of 0.4 ml/min resulted in drug retention time beyond 10 min that was more time consuming. Hence, the mobile phase was optimized at 1.0 ml/min that resulted in lower retention time around 4.8 min. Also, the less run time comparatively consumes less mobile phase solvents proving to be cost-effective during routine analysis. In this study a simple, rapid and robust method for analysis of amlodipine besylate in dissolution samples by direct injections of aqueous solutions was developed and validated. The present proposed method was compared with the reported methods in the literature shown in Table 1.

System suitability and system precision

The system suitability tests are parameters that confirm the validity of a well behaved chromatographic system. Instrument performance parameters such as peak area %RSD and USP tailing factor were established. Percent relative standard deviation (%RSD) was lower than 2%. The % RSD for mean peak area was 0.78%, mean USP tailing factor was 1.44, theoretical plates >2000. The % RSD for six replicate injections of standard was 0.54%. All the parameters tested met the acceptance criteria on all days. The result indicated that the chromatographic system is adequate for the intended analysis.

Specificity

HPLC chromatograms of blank solution (Figure 3), placebo solution (Figure 4), standard solution (Figure 5) and sample solutions obtained from dissolution testing of AMB oral films (Figure 6) indicated no interferences from the excipients with the drug peak indicating specificity of the method.

Linearity

Linearity of the method was confirmed by calibration curves for the analytical range of 20% to 150% of the standard concentration (Figure 7). A linear curve was obtained with correlation coefficient of 0.999 between analyte peak and drug concentrations. The result showed good correlation between the peak areas and concentration of the drug. The results of regression analysis of the linearity data are indicated in table 2. These data indicate that the method is linear within the specification limits.

Method precision and ruggedness

The precision of an analytical procedure expresses the closeness of the agreement (degree of scatter) between a series of measurements obtained from the multiple samples of the same homogeneous sample under the prescribed conditions. The percentage RSD obtained under different conditions was below 2%. Table 3 represents the results of intermediate and intraday precision. The relative standard deviation (RSD) of both the tests was well within the desirable limit of NMT 2.0% which is clearly indicated that the developed method is rugged.

Recovery

The accuracy was expressed as the percentage of analyte recovered by the assay method. The mean percentage recoveries (Accuracy) obtained were found between 95% to 105%.The results of recovery study are summarized in Table 4.

Robustness

The analytical method must be robust to be employed in a quality control lab. The performance of the chromatographic system and the peak response factors were not significantly influenced by the altered parameters. The change in the wavelength did not show any significant variation in peak tailing, peak area or retention time. Hence the given method was found to be robust. The result of the robustness study is indicated in Table 5.

Stationary Phase	Mobile Phase	Detection	Linearity; LOD/LOQ	Application	Reference
C18	Methanol-0.04M ammonium acetate-acetonitrile (38:38:24v/v/v) +0.02% TEA (final pH 7.1)	UV at 240 nm	2.5-100 ng/ml	Pharmacokinetic studies in rats	28
C18	Acetonitrile-methanol-pH 3.0, triethylamine solution (15:35:50 v/v/v)	UV at 237 nm	0.39-1.56 µg/ml; LOD: 0.02 µg/ml LOQ: 0.08 µg/ml	Detection of amlodipine besylate residues in swab samples	29
C18	(A) 0.04M Ammonium acetate-methanol-acetonitrile (30:30:40v/v/v); (B) 1% acetic acid-methanol (1:1 v/v)	(A) UV at 240nm; (B) MS at 2 kV soft ionization with positive mode	—	—	30
C18	1% Triethylamine (pH3.0)-acetonitrile (65:35 v/v)	UV at 220nm	75-180 ppm LOQ:21-35 ppm	Quantification of alkyl benzene sulfonates in amlodipine besylate	31
C18	Acetonitrile and 0.05M sodium dihydrogen phosphate buffer (60:40)pH 6	UV at 254 nm	amlodipine besylate: 5-30 µg/ml and telmisartan: 10-60 µg/ml	Pharmaceutical dosage form	32
C18	0.02M Potassium dihydrogen orthophosphate: acetonitrile (30:70 v/v) pH5	UV at 245 nm	Telmisartan: 32-96µg/ml Amlodipin: 4-12µg/ml	Tablet dosage form	33
C18	Buffer (0.7 % aqueous triethylamine pH 3 and methanol in the ratio of 40:60 (v/v)	UV at 239 nm	20-150 µg/ml	Pharmaceutical dosage form (Oral Film)	Present Research work

Table 1: Comparison of the performance characteristics of the present research method with the published methods.

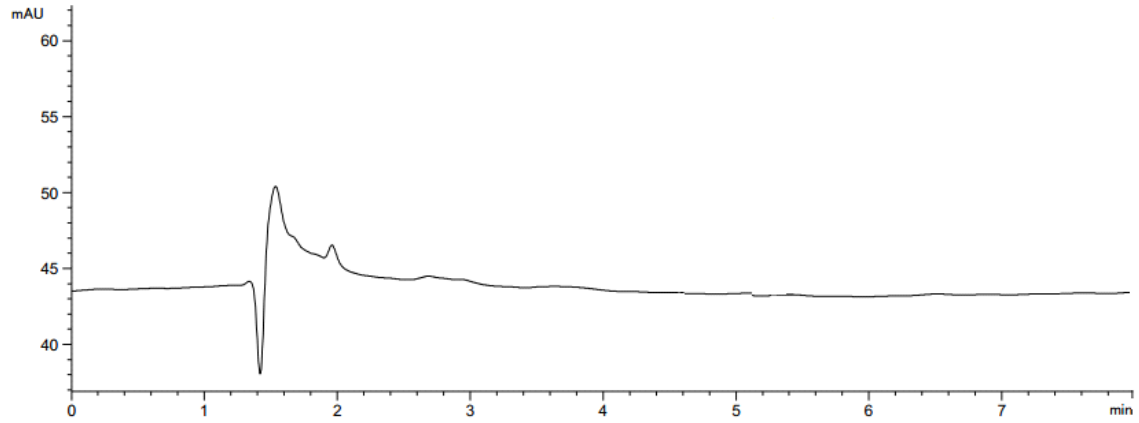

Figure 3: Chromatogram of blank solution.

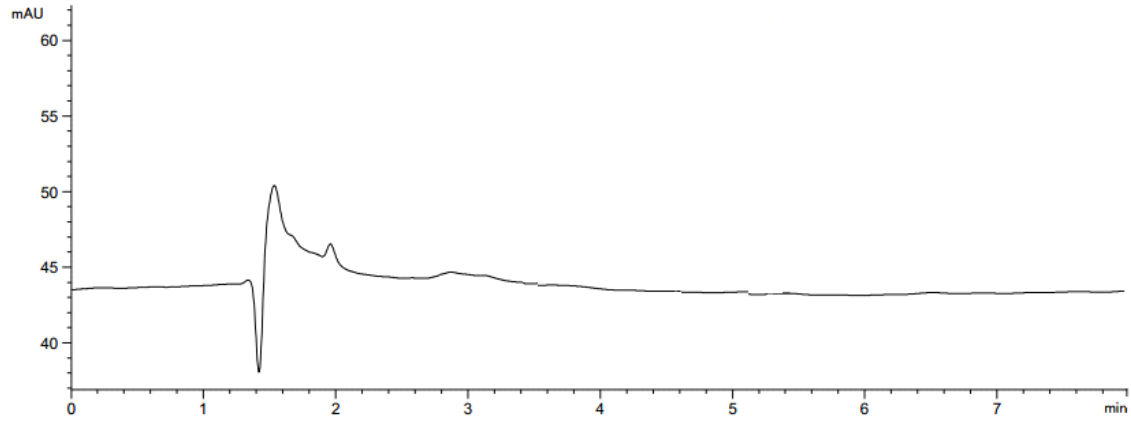

Figure 4: Chromatogram of placebo solution.

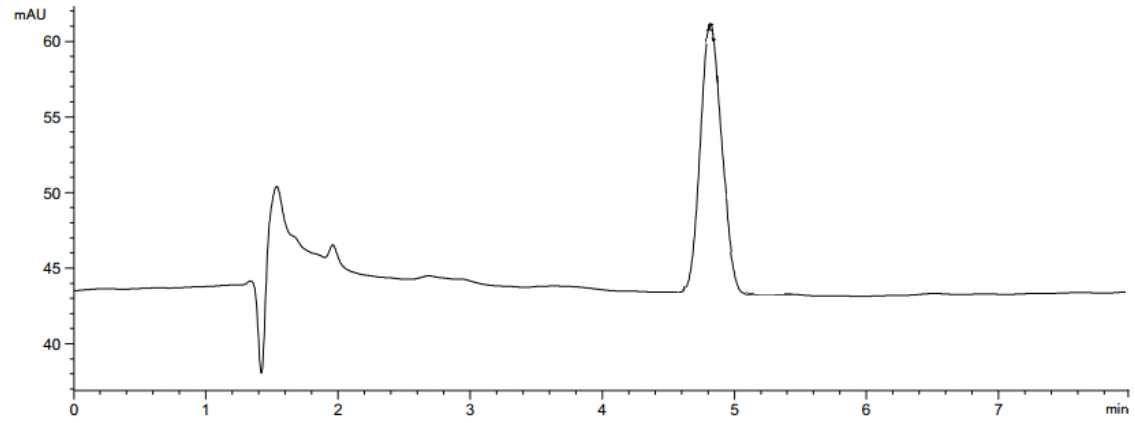

Figure 5: Chromatogram of standard AMB solution (5 µg/ml).

Solution stability

Stability studies indicated that the samples were stable when kept for 24 h. The results of the stability studies are given in Table 6.

Filter evaluation

A filter is acceptable for use if the results of the filtered portions approach 98–102% of the original concentrations of the unfiltered standard solution and the centrifuged sample solution. The results did

not show any significant variation (Table 7). The result demonstrates the absence of adsorption of AMB by the filter and therefore Whatman No. 41 filter paper is suitable in the dissolution test.

Conclusion

A simple, reproducible, isocratic HPLC method has been developed and validated for the quantitative determination of amlodipine besylate in the dissolution study of oral films using a UV detector. A complete dissolution of amlodipine besylate could be achieved after 270 seconds using USP apparatus II at 50 rpm in 500 ml of dissolution medium (0.01 M HCl). The validation results indicated that the method is specific, accurate, linear, precise, rugged and robust. The run time is relatively short which enables rapid quantification of many samples in routine analysis. Thus the developed method can be successfully employed for *in-vitro* dissolution and routine analysis of formulations containing amlodipine besylate.

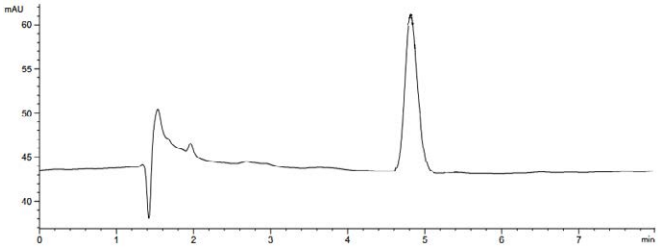

Figure 6: Chromatogram of dissolution sample solution of AMB.

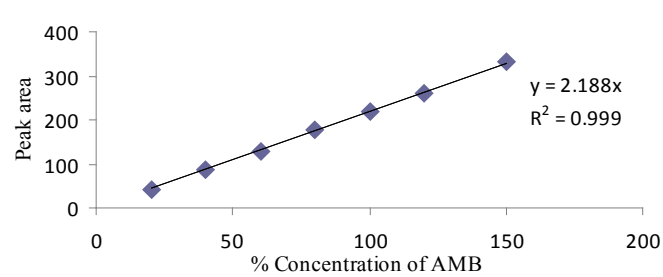

Figure 7: Calibration curve of % concentration of AMB versus peak area.

Slope	2.21
Intercept	-3.03
Correlation coefficient	0.999
n= 3	

Table 2: Results of regression analysis of the linearity data of AMB.

Replicates	% Dissolution			
	Day- 1	Day- 2	Column-I	Column-II
1	98.86	98.77	97.91	98.77
2	98.77	98.56	98.55	98.38
3	98.26	98.37	98.81	99.28
4	98.40	97.40	99.29	98.77
5	99.19	99.47	98.82	98.82
6	97.86	99.29	99.18	99.17
Mean	98.56	98.64	98.76	98.82
Standard deviation	0.475	0.740	0.496	99.17
% RSD	0.482	0.750	0.502	98.87
Mean	98.60		98.81	
Standard deviation	0.595		0.402	
% RSD	0.603		0.407	

Table 3: Results of Intermediate and Intraday Precision.

% Recovery of AMB (mean±% R.S.D)		
50%	**100%**	**150%**
98.06±0.265	99.22±0.285	98.72±0.704
n= 3		

Table 4: Results of analyte recovery.

Parameters	Variables	Mean Retention time	% RSD	Mean peak area	%RSD
Flow rate	0.8 ml	6.09	0.123	211.4	0.250
	1.2 ml	4.03	0.186	210.8	0.383
Wavelength	237 nm	4.79	0.215	216.0	0.096
	241 nm	4.8	0.204	215.6	0.301

Table 5: Result of robustness study.

	4 µg/ml	5 µg/ml	6 µg/ml
Mean	3.96	5	5.94
S.D	0.014	0.028	0.0251
%RSD	0.385	0.565	0.423
n= 3			

GC: Gas Chromatography
GC-MS: Gas chromatography coupled to Mass spectrometry
ECD: Electron Capture Detector

Table 6: Short term solution stability for AMB.

	Centrifugation (µg/ml)	Filtration (µg/ml)
Standard		
Mean	4.98	4.89
S.D	0.023	0.023
%RSD	0.463	0.471
Sample		
Mean	4.79	4.73
S.D	0.036	0.026
%RSD	0.752	0.559
n= 3		

Table 7: Filter evaluation data.

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Conflict of Interest

The author(s) declare(s) that there is no conflict of interests regarding the publication of this article.

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