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Microemulsion High Performance Liquid Chromatography Method for the Simultaneous Determination of Cetirizine HCl and Ambroxol HCl in their Tablet Formulation

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ABSTRACT

The use of microemulsions as eluents in HPLC has shown excellent potential. We have developed a novel, rapid and sensitive microemulsion HPLC (MELC) method using oil-in-water microemulsion mobilephase for the simultaneous determination of a binary mixture of cetirizine hydrochloride (CTZ) and ambroxol hydrochloride (AMB) in pure form and in their tablet formulation.
The analysis was achieved using 150 mm x 4.6 mm cyano-column. Mobile phase, containing 0.20M sodium dodecyl-sulfate, 15% propanol and 0.15 triethylamine, adjusted to pH 3.0 with orthophosphoric acid, was pumped at a flow rate of 1.0 mL min^{-1} with UV-detection at 230 nm. The method showed good linearity in the range of 1.0-10.0 µg mL^{-1} and 5-100 µg mL^{-1}, detection limit of 0.037 and 0.228 µg mL^{-1} and quantitation limit of 0.039 and 0.691 µg mL^{-1} for CTZ and ABM, respectively.
The influence of the composition of the microemulsion system was studied and the method was found to be robust with respect to some changes of the microemulsion components.

Keywords: Micro emulsion HPLC; Cetirizine HCl; Ambroxol HCl

INTRODUCTION

Cetirizine hydrochloride [$C_{21}H_{25}ClN_2O_3$,2HCl = 461.8.] (Figure 1), a piperazine derivative, metabolite of hydroxyzine and is the dihydrochloride of 2-[4-(4-chlo-robenzhydryl) piperazin-1-yl]ethoxyacetic acid. It is described as a long-acting non-sedating antihistamine with some mast-cell stabilizing activity. It appears to have a low potential for drowsiness in usual doses and to be virtually free of antimuscarinic activity. It is used for the symptomatic relief of allergic conditions including rhinitis and chronic urticaria. Cetirizine is rapidly absorbed from the gastrointestinal tract after oral doses, peak plasma concentrations being attained within about an hour [1].
Literature survey reveals that several spectrophotometric [2-6], thin layer chromatography (TLC) [7,8], high performance liquid chromatography (HPLC) [9-13], ultra-performance liquid chromatography [14] liquid chromatography-mass spectrometry (LC/MS) [15] and capillary electrophoretic [16,17] methods have been also reported for determination of CTZ from pharmaceutical formulations.

Ambroxol Hydrochloride: [$C_{13}H_{18}Br_2N_2O$.HCl =414.6.] (Figure 1) trans-4-(2-Amino-3,5-di-bromobenzylamino)cyclohexanol hydrochloride. Ambroxol is a metabolite of bromhexine and is a mucolytic used in the treatment of respiratory disorders associated with productive cough. It is given in a usual oral daily dose of 60 to 120 mg of the hydrochloride in 2 divided doses [1].

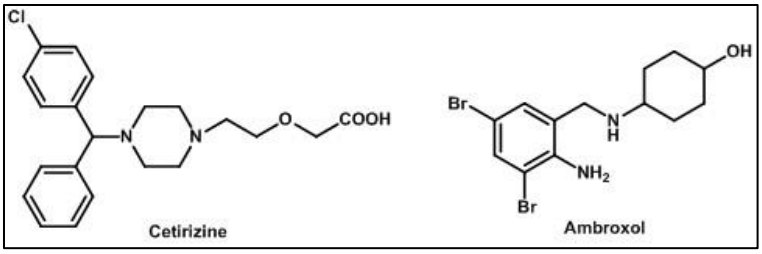

Figure 1: Structural formula of Cetirizine and Ambroxol

Literature survey reveals that several spectrophotometric [18-21], high performance liquid chromatography [22] and voltammetry [23] methods have been also reported for determination of AMB from pharmaceutical formulations.

Cetirizine and ambroxol are co-formulated in a medicinally recommended ratio of 1:12. Several methods were reported for the simultaneous determination of CTZ and AMB in their co-formulated tablets such as spectrophotometry [24-27], ultra-performance liquid chromatography [28] and high performance liquid chromatography [29-33].

Microemulsion- HPLC is a recent development offering reduced sample preparation times for complex samples and generic separation conditions applicable to a wide range of solutes [34].

Microemulsion liquid chromatography (MELC) is an extension of micellar liquid chromatography (MLC). In MLC a surfactant is added in excess of the critical micelle concentration (CMC) with the result that the mobile phase contains a large amount of micelles. The micelles affect the chromatography as analytes partition with the micelles rather than adsorb onto the stationary phase [34].

Microemulsions are stable, isotropically clear solutions consisting of an oil (such as octane) and water stabilized by a surfactant and co-surfactant. To form such a mixture, the interfacial tension between the oil and water has to be decreased by the addition of both a surfactant (e.g. sodium dodecyl sulfate (SDS)) and a co-surfactant (e.g. medium chain alcohols, such as butanol or pentanol). The use of microemulsions as a mobile phase changes the characteristics of the separation and has been widely used due to its unique power of dissolving microemulsions of oil in water for hydrophobic compounds. In addition, as a mobile phase it is well combined with a reversed phase column. The elution order of compounds in this method is often coincides with their order of elution for the classical reversed phase HPLC [34]. Ryan R. *et al* [34] introduce the concepts of MELC and discuss the possible benefits and future applications.

Recently several papers concerning the general applicability of microemulsion as the eluent in HPLC have been reported [35-37] proving the excellent potential of such an application.

The objective of the study

The aim of the present work was to develop an efficient and novel liquid chromatographic method using microemulsion as mobile phase for the rapid simultaneous determination CTZ and AMB in pharmaceutical preparations, in a single chromatographic run.

Although there are a several methods for their simultaneous determination, it was the first time to use the microemulsion as a new mobile phase and also it is the first time to use the cyano column as stationary phase which is adopted for the mixture separation instead of reversed stationary phase (C18 column) that has been used by other reported HPLC methods [29-33]

EXPERIMENTAL SECTION

Materials and reagents

All the chemicals used were of Analytical Reagent grade, and the solvents were of HPLC grade.

Cetirizine Hydrochloride and Ambroxol hydrochloride were kindly provided by Chemipharm Pharmaceutical Industries S.A.E (6^{th} October City, Egypt). The purity of Cetirizine Hydrochloride was 99.85 % and that of Ambroxol hydrochloride was 99.90 %. They were used as received without further purification.

Cetzine A - Tablet; Batch No. 250001 (Glaxo Smithkline Pharmaceuticals Ltd) was purchased from commercial sources in the pharmacy. Each tablet labeled to contain 5 mg Cetirizine Hydrochloride and 60 mg ambroxol hydrochloride.

Sodium dodecyl sulfate (SDS) 99% purity was obtained from Park Scientific Limited, Northampton, UK. 1-Propanol, methanol and di-isopropyl ether (all of HPLC grade) as well as triethylamine (TEA) were obtained from Riedel-deHäen (Seelze, Germany). 1-Butanol and tetrahydrofuran (HPLC grade) were obtained from Merck (Darmstadt, Germany). 1-Octanol (HPLC grade) was obtained from Aldrich (Gillingham,UK). 1-Butyl acetate was obtained from Fluka (Buchs, Switzerland). Orthophosphoric acid for analysis was obtained from Prolabo (Paris, France).

Apparatus

MELC separation was performed with Shimadzu^{TM} LC-20A series chromatograph equipped with a 20 µl Rheodyne injector valve and a SPD-20A UV detector operated at 270 nm. LC workstation (Nishinokyo-Kuwabaracho, Nakagyo- Ku, Kyoto, Japan).

Columns and mobile phase

Separation was achieved on a shim-pack cyano column (150 mm×4.6mm i.d., 5 µm particle size 100 Å) from Shimadzu. a reversed phase analytical column C-8 (250 × 4.6 mm) 5 µm (Kromasil), was used for the reported reference method. The columns were operated at ambient temperature. The components of the microemulsion were 0.2 M SDS, 15 % 1-propanol, 1 % 1-octanol and 0.3 % TEA in 0.02 M phosphoric acid, pH = 3.0. All the microemulsion components were mixed together and the pH was adjusted using TEA. Then the mixture was treated on an ultrasonic bath for 30 min. The resulting transparent mobile phase was filtered through a 0.45 µm membrane filter (Millipore, Ireland). Microemulsion was stable for at least 2 months.

Sample preparation and procedures

Standard solutions of CTZ and AMB (1000 µg/mL) were prepared in methanol. CTZ standard solution was further diluted with methanol to obtain solution of 100 µg/mL concentration. The standard solutions were found to be stable for at least one week when kept in the refrigerator at 4°C.

General procedures and construction of calibration graphs: To a set of 10 mL volumetric flasks, increasing volumes of the standard solutions of CTZ and AMB were quantitatively transferred so as to give solutions containing the two drug substances within the concentration range of 1.0-10.0 and 10.0-100.0 µg/mL, respectively, after being diluted to 10.0 mL with the mobile phase. Injection into the HPLC system was performed at ambient temperature (25 ºC). Twenty microliter aliquots were injected (in triplicate) and the calibration curves were constructed by plotting the peak height against the final concentration of both drugs. Alternatively, the corresponding regression equations were derived.

Application of the proposed methods to the determination of the studied drugs in synthetic mixtures: Accurately measured aliquots of the working standard solutions of both drugs were transferred into a series of 10 mL volumetric flasks to prepare different synthetic mixtures of CTZ and AMB in the ratio of 1:12. The solutions were then diluted with either the mobile phase to the volume, mixed well and analyzed as described under construction of the calibration graphs. The concentration of each drug was determined using, either the calibration curve or the corresponding regression equation.

Application of the proposed methods to the determination of the studied drugs in co-formulated tablets: Ten Cetzine A tablets were accurately weighed, finely pulverized, and thoroughly mixed. An accurately weighed amount of pulverized tablets equivalent to 8.333 mg CTZ and 100.0 mg AMB (according to their pharmaceutical ratio) was transferred into small conical flask and extracted with 3 x 30 mL of methanol. The extracts were collected then filtered into 100 mL volumetric flask. The conical flask was washed with few milliliters of methanol. The washing was passed into the same volumetric flask, and then the flask was made up to volume with same solvent. The solution filtered through 0.45 µm sample filters (RC25, Sartorius AG, Goettingen, Germany). Aliquots covering the working concentration range cited in table 1 were transferred into 10 mL volumetric flasks. Proceed as described under "Construction of calibration graph. The nominal content of the tablets was calculated using the corresponding regression equation

RESULTS AND DISCUSSION

Development of the method

The different parameters affecting the separation selectivity of the MELC system have been investigated and optimized. Using a mobile phase consisting of 0.20 M SDS, 15 % 1-propanol, 1 % 1-octanol and 0.3 % TEA in 0.02 M phosphoric acid of pH 3.0, an optimum separation of the two drug substances, with a resolution factor of 3.75, was achieved in a reasonable time less than 5 min, with maximum detector response.

Table 1: Effect of the experimental parameters on the NTP and resolution

Parameters	NTP		Rs
	CTZ	AMB	
Concentration of surfactant (M)			
0.1	1500	2100	3.24
0.15	1663	2140	3.58
0.2	1740	2540	3.78
0.25	1632	2060	3.35
Concentration of Co surfactant (%)			
7.5	1000	1980	2.85
10	1500	2156	3.16
12.5	1652	2395	3.4
15	1740	2540	3.78
17.5	1755	2550	3.78
Type of Co surfactant			
Propanol	1740	2540	3.78
Butanol	2316	1501	3.74
THF	2265	1355	3.64
Acetonitril	1858	942	2.8
Type of internal phase			
Octanol	1740	2540	3.78
Di-isopropyl ether	1254	2390	3.6
Ethylacetoacetate	1200	2156	4
pH of the mobile phase			
2.5	1675	2400	3.88
3	1740	2540	3.78
4	1564	2354	3.42
5	1358	2100	2.8
6	Poor resolution		
6.5	Overlapped peaks		

Figure 2 represents a typical chromatogram of AMB and CTZ. The retention times for AMB and CTZ were 3.3 and 4.7 min., respectively. The different parameters affecting the separation selectivity of the MELC system have been investigated and optimized (Table 1).

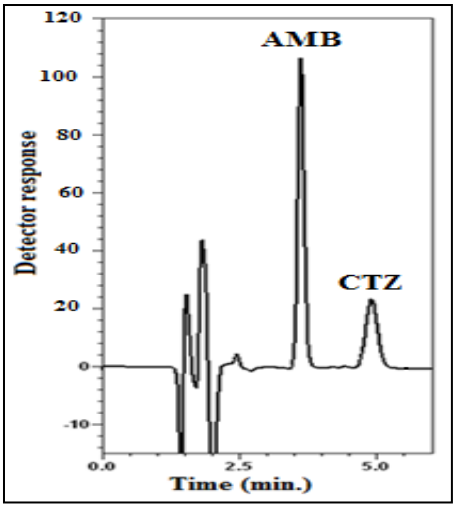

Figure 2: Typical chromatogram for the separation of AMB (90 µg/mL,3.3 min) and CTZ (9 µg/mL,4.7 min) using microemulsion mobile phase. Chromatographic system: column, cyano(5µm) 150 mm× 4.6 mm. Mobile phase microemulsion, 0.2 M SDS, 15 % n-propanol, 1% n-octanol, 0.3% triethylamine, in 0.02 M phosphoric acid, pH 3.0. Flow rate, 1 mL/min, UV detection at 230 nm; column temperature, ambient

The concentration of the surfactants

The effect of SDS concentration on retention time and peak efficiency represented as number of theoretical plates (NTP) was investigated using microemulsions containing SDS concentrations ranged from 0.10 to 0.25 M. It was found that an increase in the concentration of SDS decreased the retention time of both substances over the investigated range due to their distribution into the increased volume of the microemulsion droplets or to the surface of the droplets which run with the speed of the mobile phase (Figure 3).

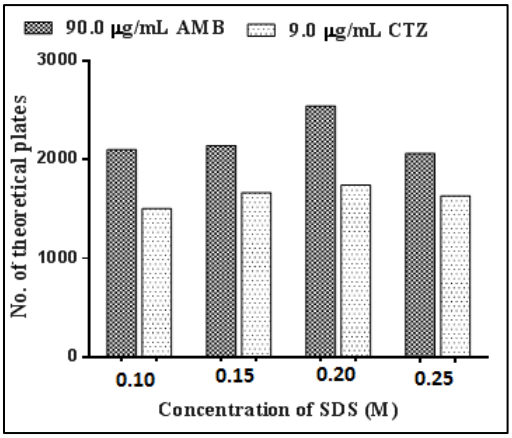

Figure 3: Effect of molar concentration of surfactant (SDS) on the number of theoretical plates of AMB and CTZ using microemulsion mobile phases consisting of different concentrations of SDS, 15 % n-propanol, 1 % n-octanol, 0.3 % triethylamine, in 0.02 M phosphoric acid, pH 3.0

Meanwhile, increasing SDS concentration increased the peak efficiency of both drugs up to 0.20 M as indicated by increased NTP; further increase in SDS concentration up to 0.25 M slightly decreases NTP. A concentration of 0.20 M was found to be suitable for routine use as it provides adequate elution time and peak efficiency.

The effect of co-surfactant

The co-surfactant nature greatly influences the mobile phase behavior and changing the type of the co-surfactant can alter the selectivity [34] 1-Butanol, propanol, tetrahydrofuran and acetonitrile were investigated in an attempt to study the effect of the nature of the co-surfactant on the efficiency of

separation. The NTP of the two drugs are given in figure 4 as a function of the co-surfactant investigated. Propanol provided excellent efficiency of the two peaks, butanol and tetrahydrofuran provided reasonable efficiency of the two peaks, while the use of acetonitrile decreases the column efficiency for separation as indicated by the decrease in the number of theoretical plates.

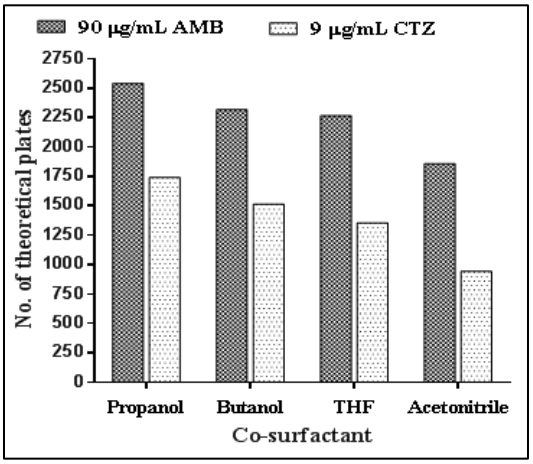

Figure 4: Effect of co-surfactant on the number of theoretical plates of AMB 90 µg/mL and CTZ 9 µg/mL using microemulsion mobile phases consisting of 0.2 M SDS, 15 % different co-surfactant, 1 % n-octanol, 0.3 % triethylamine, in 0.02 M phosphoric acid, pH 3.0

The effect of propanol concentration was investigated over the concentration range 5–17.5%. It was found that, increasing the co-surfactant concentration results in decreasing the retention times of both drugs due to increasing the proportion of organic phase in the microemulsion. 15% propanol was selected as optimum.

The internal organic phase

A micellar mobile phase identical to the microemulsion system but without the internal phase n-octanol, was investigated. It was found that the resolution of the peaks as well as peak area and number of theoretical plates were decreased. Three different organic solvents 1-octanol, butyl acetate and di-isopropylether were tested as internal organic phases (1%) so as to present a range of polarity. The molecular volume of the oil, relative to the hydrophobic chain of the surfactant, affects the extent to which it penetrates the surfactant tails of the oil water interface. It was found that the separation could be successfully achieved using each of the three solvents. However, 1-octanol seemed to be optimal for separation and detection of both analytes because it provides the best peak area, retention time (Figure 5) and number of theoretical plates.

The effect of pH

The pH of the mobile phase was changed in intervals from 2.4 to 6.5 using increasing amounts of triethylamine in phosphoric acid. The retention factors of the two drugs were plotted against different pH values. It was found that the retention time of CTZ gradually decreased upon increasing the pH value due to increased ionization to carboxylate anion (pK_a = 2.9) [38]. Meanwhile, the retention time of AMB was not significantly affected till pH 6.0 as it will be fully ionized over the investigated pH range (pK_a = 6.84) [38]. CTZ has log P value of 1.62, while AMB has log P value of 3.0. The two drugs differ in hydrophobicity and dissociation constants as expressed by their log P and *pKa* values, respectively.

In this study, a pH value of 3.0 seemed to be optimal for the separation of both analytes as it provides satisfactory resolution (R_s = 3.78) in short chromatographic run (5 min.) and good peak efficiency as indicated by NTP. However, upon increasing the pH, the resolution was decreased due to ionization of CTZ producing the carboxylate anion till complete overlap of the two peaks at pH 6.5.

The flow rate

The effect of flow rate on the formation and separation of peaks of the studied compounds was studied in the range of 0.5 - 1.5 mL/min. A flow rate of 1 mL/min. was optimal for good separation in a reasonable time (5 min.).

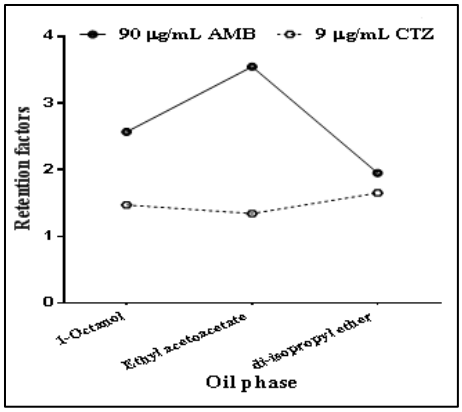

Figure 5: Effect of oil phase on the retention factors of AMB 90 µg/mL and CTZ 9 µg/mL using microemulsion mobile phases consisting of 0.2 M SDS, 15 % n-propanol, 1 oil phase, 0.3 % triethylamine, in 0.02 M phosphoric acid, at pH 3.0

DISCUSSION

The UV spectra of CTZ solution in methanol absorption maximum at 231 nm. and AMB showed absorption maxima at 244 and 310 nm (Figure 6). Thus, conventional UV spectrophotometry can't be used for the simultaneous determination of both drugs. Thus, the proposed method was suggested for their simultaneous determination without any interference.

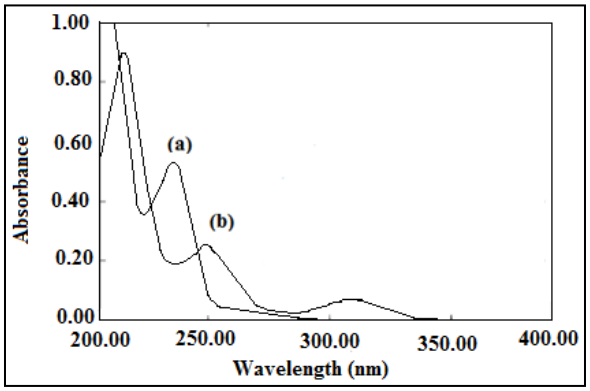

Figure 6: Absorption spectra of (a) CTZ (5.0 µg/mL) and (b) AMB (5.0 µg/mL) in methanol

The proposed method allowed satisfactory resolution of both drugs; capacity factor "k" was 2.1 and 1.2, tailing factor "T" was 1.17 and 1.08 for CTZ and AMB, respectively, resolution factor (R_s) = 3.78 and selectivity factor (α) = 1.75) in a reasonable time less than 5 min. The retention times for AMB and CTZ were 3.3 and 4.5 min., respectively. The proposed MELC method offers high sensitivity about 0.228 µg/mL of AMB and 0.037 µg/mL of CTZ could be detected accurately. It also permitted the quantification of the drugs in co-formulated tablets in a short time run with high sensitivity.

Validation of the proposed methods

The developed analytical methods were then subjected to method validation according to ICH Q2(R1) guidelines [39]. The following parameters were considered: linearity, sensitivity, LOD, LOQ, specificity, accuracy and precision.

Linearity

Linear relationships were established for both drugs by plotting the either the peak area or derivative amplitude against each drug concentration. Linear relationships were obtained over the concentration range

cited in table 2. Linear regression analysis of the data by the proposed methods gave the following equations:

PH = 0.343+ 3.048 C (r =0.9999) for CTZ, n = 10
PH = -5.840 + 2.247 C (r =0.9999) for AMB, n = 10

Where PH is the peak height, "C" is the concentration of the drug (µg/mL), "r" is correlation coefficient and "n" is sample numbers.
Statistical analysis of the data gave high values of the correlation coefficients (r) of the regression equations, small values of the standard deviation of residuals ($S_{y/x}$), intercepts (S_a), and slopes (S_b), and small values of the percentage relative standard deviations and the percentage relative errors (Table 2). These data points to low scattering of points around the calibration curves and the high accuracy and precision of the proposed methods.

Table 2: Performance data for the determination of the studied drugs by the proposed methods

Parameter	CTZ	AMB
Conc. range (µg/mL)	1.0-10.0	10.0-100.0
Correlation coefficient	0.9999	0.9999
Slope	3.048	2.246
Intercept	0.343	-5.84
LOD (µg/mL)	0.038	0.228
LOQ (µg/mL)	0.114	0.691
Sy/x	0.104	0.711
Sa	0.035	0.155
Sb	0.011	0.007
% RSD	0.944	1.211
% Er	0.299	0.366

Limit of Quantification (LOQ) and Limit of Detection (LOD)
LOQ and LOD were calculated according to ICH Q2(R1) recommendations [39] using the following equations and the results were presented in table 2:

$$LOQ = 10\ S_a/b \text{ and } LOD = 3.3\ S_a/b$$

Where S_a = standard deviation of the intercept and b = slope of the calibration curve.

Accuracy and Precision
To prove the accuracy of the proposed methods, the results of the assay of AMB and CTZ were compared with those of the reference method [30]. Statistical analysis of the results using Student's *t*-test and variance ratio *F*-test [40] revealed no significant difference between the performance of the proposed and reference methods regarding the accuracy and precision, respectively (Table 3).
The reference method depends on the simultaneous determination of AMB and CTZ by HPLC: The chromatography system used a reversed phase C-8 column with UV- Vis detection at 230 nm. Mobile phase consisted of acetonitrile – 0.1% triethlamine (50:50 v/v) (pH adjusted to 4.0 using 10% ortho phosphoric acid) at a flow rate of 1.5 mL/min using propranolol as internal standard (I.S.). The. The intra-day and inter-day precisions and accuracy of proposed methods were examined by triplicate analysis of AMB and CTZ at three different concentrations in one day and for three consecutive days. The precision of the proposed method was satisfactory, as indicated by the low values of SD and RSD, also the low values of % Er indicate good accuracy of the method (Table 4).

Specificity
The specificity of the methods was investigated by observing any interference encountered from the presence of tablet excipients such as microcrystalline cellulose, lactose, pre-gelatinised starch and magnesium stearate. These excipients did not interfere with the proposed methods.

Table 3: Assay results for the determination of the studied drugs in pure form by the proposed and reference methods

Parameter	Proposed method		Reference method (30)	
	CTZ	AMB	CTZ	AMB
	99.99	100.3	100.1	100.2
± SD	± 0.298	± 0.366	± 0.304	± 0.306
t-value	0.15 (2.14)	0.27 (2.14)		
F-value	1.04 (4.735)	1.43 (4.735)		

Each result is the mean recovery of three separate determinations n = 10
Figures between brackets are the tabulated t and F-values at (P= 0.05).

Table 4: Accuracy and precision data for the determination of CTZ and AMB in pure form by the proposed methods

Conc. (µg/mL)		CTZ			AMB		
		3	6	9	30	60	90
Intra-day	$\bar{X}$	100.32	99.85	100.14	99.85	100.1	99.85
	± SD	0.56	0.29	0.43	0.8	0.35	0.8
	% RSD	0.56	0.29	0.43	0.8	0.35	0.8
	% Error	0.32	0.17	0.25	0.46	0.2	0.46
Inter-day	$\bar{X}$	100.69	99.67	100.23	100.37	100.68	100.37
	± SD	0.58	0.21	0.47	0.8	0.53	0.8
	% RSD	0.58	0.21	0.47	0.79	0.53	0.79
	% Error	0.33	0.12	0.27	0.46	0.3	0.46

Each result is the mean recovery of three separate determinations

Robustness

The robustness of the proposed method was demonstrated by the constancy of the fluorescence intensity with the deliberated changes in the experimental parameters such as surfactant concentration (0.20 ± 0.01M), co-surfactant concentration (15.0 ± 1.0%), organic modifier concentration (1.0 ± 0.1%) and pH (3.0 ± 0.1) doesn't affect the analytical procedure as revealed during parameters study

Analysis of AMB/CTZ in synthetic mixtures and co-formulated tablets

The proposed methods were applied to the simultaneous determination of AMB and CTZ in synthetic mixtures in the medicinally recommended ratio of 12:1. Furthermore, the proposed methods were successfully applied to their determination in co-formulated tablets. The results shown in tables 5 and 6 are in good agreement with those obtained using the reference method [30]. Statistical analysis of the results obtained using Student's *t*-test and variance ratio *F*-test revealed no significant difference between the performance of the proposed and reference methods regarding the accuracy and precision, respectively.

Table 5: Assay results for the determination of the studied drugs in their synthetic mixture

Parameter	Proposed method		Reference method (30)	
	CTZ	AMB	CTZ	AMB
$\bar{X}$	100.31	100.3	100.4	100.4
± SD	± 1.22	0.416	± 0.828	± 0.644
t-value	0.38 (2.78)	0.11 (2.78)		
F-value	2.17 (19.00)	2.40 (19.00)		

Each result is the mean recovery of three separate determinations, n =3
Figures between brackets are the tabulated t and F-values at (P= 0.05).

Table 6: Assay results for the determination of the studied drugs in their co-formulated Cetzine-A® tablets

Parameter	Proposed method		Reference method (30)	
	CTZ	AMB	CTZ	AMB
$\bar{X}$	99.9	99.76	100.2	100.2
± SD	± 0.263	± 0.344	± 0.687	± 0.635
t-value	0.46 (2.78)	0.62 (2.78)		
F-value	6.84 (19.0)	3.41 (19.0)		

Each result is the mean recovery of three separate determinations, n =3
Figures between brackets are the tabulated t and F-values at (P= 0.05).

CONCLUSION

A very specific and sensitive MELC was developed for the simultaneous determination of AMB and CTZ in pure form and in their tablet dosage forms in a short chromatographic run (5 min). The LOD and R.S.D. values are sufficiently good for the applicability of these methods for routine quality control laboratories.

CONFLICT OF INTERESTS

The author doesn't have direct financial relation with the trademarks mentioned in this paper, so he has no conflict of interests.

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