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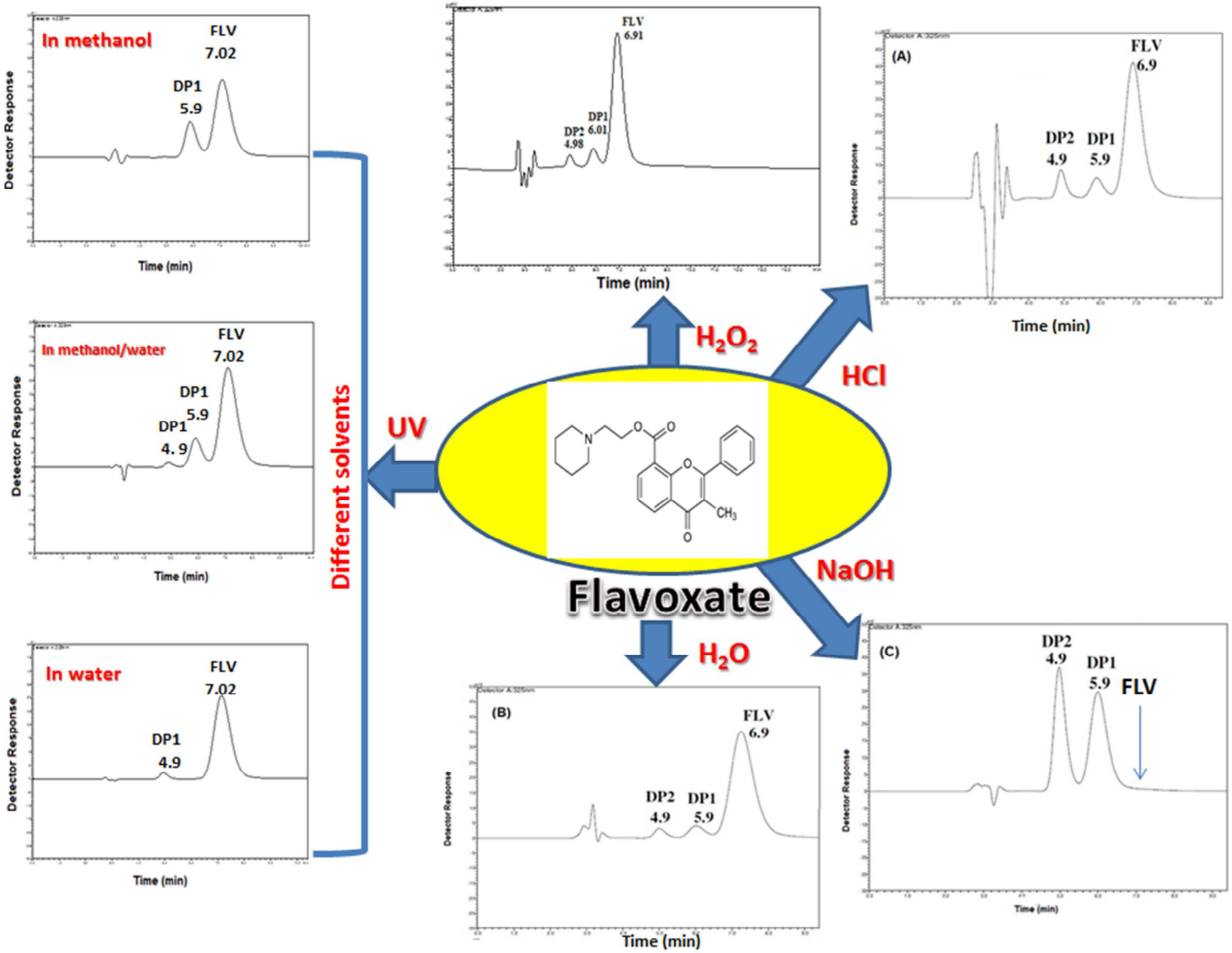
First micellar HPLC method for the assay and comprehensive study of degradation behavior of flavoxate HCl as *per* ICH guidelines.



A Green HPLC Method for the Analysis and Stability Study of Flavoxate HCl using Micellar Eluent

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Abstract

An accurate, reliable and environmentally benign stability-indicating micellar liquid chromatographic method was developed and validated for the determination of flavoxate HCl (FLV) in presence of its stress induced degradation products. Good resolution of FLV from its degradation products was achieved using a reversed phase BDS Hypersil phenyl column (4.6 mm × 250 mm, 5 µm particle size) with a micellar mobile phase consisting of 0.15 M sodium dodecyl sulphate, 15% *n*-propanol, 0.3% triethylamine and 0.02 M orthophosphoric acid (pH 2.5). UV quantitation was set at 325 nm. The linear regression analysis data for the calibration plot of FLV showed a good linear relationship over the concentration range of 2.0-40.0 µg mL^{-1} with lower detection limit of 0.40 µg mL^{-1}. Stability of FLV was investigated as *per* ICH-prescribed stress conditions including acidic, alkaline, neutral, oxidative and photolytic conditions. Significant degradation of FLV was observed under all studied stress conditions. A kinetic study was conducted to investigate the oxidative degradation of FLV at different temperature settings; reaction rate constants, half-life times and activation energy were calculated. A proposal for the degradation pathways was also postulated. The proposed method was applied for the assay of FLV in its commercial tablets with mean percentage recovery of 99.80 ± 1.41. Statistical comparison of the results of the proposed method with those obtained by the comparison method revealed no significant differences in the performance of the two methods regarding accuracy and precision.

Keywords: Flavoxate HCl, MLC, Stability-indicating Assay, Tablets.

Introduction

Flavoxate hydrochloride(FLV), 2-piperidinoethyl 3-methyl-4-oxo-2-phenyl-4H-chromene-8-carboxylate hydrochloride, Fig. 1) belongs to a series of flavone derivatives, which exhibit strong smooth muscle relaxant activity with selective action on the pelvic. It has anticholinergic and antimuscarinic effects; it is used for the symptomatic relief of pain, urinary frequency and incontinence associated with inflammatory disorders of the urinary tract. FLV is also used for the relief of vesicourethral spasms resulting from instrumentation or surgery.[1]

FLV is the subject of a monograph in the British Pharmacopoeia [2] that recommends a non-aqueous titrimetric method for its determination in pure form using perchloric acid as a titrant and a spectrophotometric method for its determination in tablets, in which its absorbance was measured at 293 nm in 0.1 M HCl. A good guide to the analytical methods published for the assay of FLV in pharmaceutical preparations and biological fluids until 2001 was presented as a monograph in the series of *"Analytical Profiles of Drug Substances and Excipients"* [3]. Recently, some analytical methods were developed for determination of FLV such as spectrophotometry, [4, 5] potentiometry [6] and HPLC. [7, 8] Also, some HPLC methods focused on the analysis of FLV active metabolite (3-methylflavone-8-carboxylic acid) in biological fluids. [9, 10]

Literature survey revealed that; only one stability–indicating HPLC method was reported for FLV. [7] This method was concerned only with the study of alkaline and acidic degradation of FLV using acetonitrile-12 mM ammonium acetate (45:55 v/v, pH 4.0) as a mobile phase with UV detection at 220 nm and flow rate of 1.5 mL min^{-1}. Moreover, a potentiometric method [6] based on ion selective electrodes was applied for the analysis of FLV in presence of its degradation product. However, in this method the behavior of FLV under different stress conditions was not studied. So, it was essential to

develop a rapid, simple and cost-effective HPLC method to study the degradation behavior of FLV under different stress degradation conditions as recommended by ICH guidelines. [11, 12]

Recently, micellar liquid chromatography (MLC) has received much attention due to its advantages and capabilities, such as simultaneous separation of charged and uncharged solutes, unique separation selectivity, robustness, high reproducibility, low cost and safety of analysis. [13] The presence of a surfactant not only modifies the interactions established inside the column but also reduces the necessary amount of organic solvent in the mobile phase. The low organic solvent contents (6-15%, v/v) in comparison with those needed in classical RP-LC results in reduced cost and hazard effect of organic solvents, which may become prominent for *"green chemistry"*. Also, the stabilization of the organic solvent in the micellar medium decreases the risk of evaporation. [14]

The present study is aimed to develop a stability-indicating assay method to study the degradation behavior of FLV under a variety of degradation conditions including acidic, alkaline, neutral, oxidative and photolytic conditions adopting MLC technique. Also, it is aimed to apply the proposed method for quality control of FLV in commercial tablets. Herein, MLC approach was adopted to develop a green methodology with minimum toxicity to the analyst and the environment in addition to the high resolution power and selectivity achieved by this technique.

Experimental

Apparatus

- *HPLC system*: separation was achieved with a Shimadzu HPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with an LC-20AD delivery system, a Rheodyne injector valve with 20 µL loop and a SPD-20A UV-Visible detector. Mobile phases were degassed using a DGU-

20A5 online solvent degasser. The apparatus was interfaced to a computer for data acquisition with a CBM-20A communication bus module.

- *pH-Meter*: A Consort P901 pH meter (Belgium) was used for pH-adjustment.
- *CAMAG UV-lamp* (S/N 29000), dual wavelength (254/366), 2×8 W, (Muttenz, Switzerland) was used in the photo-stability study.

Materials

- Flavoxate HCl pure sample with certified purity of 99.0% was kindly provided by Amoun Pharmaceutical Co, Cairo, Egypt.
- Genurin S.F® 200 tablets labeled to contain 200 mg flavoxate HCl/tablet (product of Medical Union Pharmaceuticals, Abu Sultan, Ismailia, Egypt) was purchased from a local pharmacy.

Chemicals and Reagents

All solvents used were of HPLC grade and chemicals were of analytical reagent grade:

- Methanol, *n*-propanol (HPLC grade) and sodium dodecyl sulfate (SDS, 99%) were purchased from Sigma-Aldrich Co. (Chemie Gmbh, Munich, Germany).
- Triethylamine (TEA) and orthophosphoric acid (85% w/v) were purchased from Riedel-deHäen (Seelze, Germany).
- Sodium hydroxide was purchased from Winlab (Middlesex, UK).
- Hydrochloric acid (32% w/v) and hydrogen peroxide (30% w/v) were purchased from El-Nasr Pharm. Chem. Co. (Cairo, Egypt).

Chromatographic conditions

- *Column:* BDS Hypersil phenyl column (4.6 x 250 mm, 5 µm particle size), Thermo Electron Corporation, Runcorn, UK.
- *Mobile phase:* A micellar mobile phase containing 0.15 M SDS, 15% *n*-propanol, 0.3% TEA and 0.02 M orthophosphoric acid. pH of the mobile phase was adjusted to pH 2.5 with orthophosphoric acid . The mobile phase was filtered through 0.45 µm Millipore membrane filter and degassed by sonication for 30 min before use.
- *Flow rate:* 1 mL/min
- *UV/VIS detector wavelength:* 325 nm.

Preparation of standard solution

A standard solution containing 400.0 µg mL^{-1} of FLV was freshly prepared in methanol and protected from light.

General recommended procedures

Construction of calibration graph

Working solutions containing 2.0-40.0 µg mL^{-1} of FLV were prepared by serial dilution of the standard solution with the mobile phase. Twenty µL aliquots were injected (triplicate) and eluted with the mobile phase under the optimum chromatographic conditions. The average peak areas of FLV were plotted *versus* the corresponding drug concentrations (µg mL^{-1}) to obtain the calibration graph and the regression equation was derived.

Assay of Tablets

Ten Genurin S.F® 200 tablets were accurately weighed; finely pulverized and thoroughly mixed. An accurately weighed amount of the powder equivalent to 40.0 mg of declared active principle was transferred into 100 mL volumetric flask. About 90 mL of methanol was added and the

mixture was sonicated in an ultrasonic bath for 30 min. The volume was completed to the mark with the same solvent, mixed well and filtered with 0.45 µm cellulose acetate syringe filter. Accurately measured aliquots of the filtrate were transferred into a series of 10 mL volumetric flasks, diluted to the mark with the mobile phase and mixed well to obtain different concentrations within the working concentration range. Twenty µL aliquots were injected (triplicate) and eluted with the mobile phase under the optimum chromatographic conditions. The nominal contents of the tablets were calculated from the corresponding regression equation.

Procedures for forced degradation studies

- *Acidic, alkaline and neutral degradation*

Aliquots of FLV standard solution (containing 400.0 µg) were transferred into three small conical flasks; 5 mL aliquots of 0.1 M NaOH, 1.0 M HCl and distilled water, respectively were added. The alkali treated solution was kept at room temperature for 10 min, the acid treated solution was heated in a boiling water bath for 30 min, where the solution subjected to neutral degradation was heated at 80 ºC in a thermostatically controlled water bath for 30 min. At the specified time, the contents of the flasks were cooled, neutralized to pH 7.0 using either 0.1 M HCl or 1.0 M NaOH (except the solution under neutral degradation) and the solutions were then transferred into a series of 10 mL volumetric flasks. The volumes were completed with the mobile phase and the solutions were mixed well. Triplicate 20 µL injections were made for each sample.

- *Oxidative degradation*

Aliquots of FLV standard solution (containing 400.0 µg) were transferred into a series of small conical flasks and 2 mL of H_2O_2 solution (30% w/v) were added to each flask. The solutions were heated in a

thermostatically controlled water bath at different temperature settings (60, 70, 80 °C) for different time intervals (10-40 min). At the specified time, the contents of each flask were cooled. The solutions were transferred into a series of 10 mL volumetric flasks, the volumes were completed with the mobile phase and the solutions were mixed well. Triplicate 20 µL injections were made for each sample.

- *Photolytic degradation*

FLV solutions (400.0 µg mL^{-1}) were prepared in different solvents (methanol, methanol: water (1:1 v/v) and water) and exposed to UV-light at a wavelength of 254 nm at a distance of 15 cm placed in a wooden cabinet for 3 hrs. At the specified time, 1.0 mL of each solution was transferred into 10 mL volumetric flask, completed to the mark with the mobile phase and mixed well. Triplicate 20 µL injections were made for each sample.

Results and Discussion

A rapid and sensitive liquid chromatographic method was developed and validated as a suitable tool for the quality control and study of FLV stability using micellar mobile phase as a green eluent. The development of a stability-indicating assay method of FLV was necessary to study its degradation behavior under different stress conditions. Only one HPLC method [7] was reported to investigate its alkaline and acidic degradation. In this method a mobile phase with a large proportion of organic solvent was used (acetonitrile-12 mM ammonium acetate, 45:55 v/v at a flow rate of 1.5 mL min^{-1}) with UV detection in the near UV region at 220 nm which may introduce some interferences. This leads us to extend this study to investigate the oxidative and photolytic degradation behavior of FLV to help understanding its complete degradation profile. The proposed method has the ability to resolve FLV from all degradation products generated under different

stress conditions including alkaline, acidic, neutral, oxidative and photolytic conditions. It was also applied to investigate the kinetics of oxidative degradation of the drug. The applicability of the method was extended to determine FLV in its commercial tablets. The proposed method has the advantages of being simple and environmentally benign due to the use of small amount of organic solvents compared to conventional hydro-organic LC methods. Currently, the use of MLC found pronounced applications due to its agreement with the "*green chemistry*" concept.

Optimization strategy and mobile phase selection

Extensive experimental studies were carried out to select the most efficient parameters for the analysis. The final experimental conditions were chosen after testing the type of stationary phase, composition of the mobile phases and detection wavelength.

Choice of column

For selection of the suitable stationary phase for separation of FLV from its forced degradation products, three columns were investigated including:

1. CLC Shim-pack C_8 column (4.6 mm x 250 mm, 5 µm particle size), Shimadzu Corporation, Japan.
2. CLC Shim-pack CN column (4.6 mm x 150 mm, 5 µm particle size), Shimadzu Corporation, Japan.
3. BDS Hypersil phenyl column (4.6 mm x 250 mm, 5 µm particle size), Thermo Electron Corporation, Runcorn, UK.

The phenyl column was found to be the most suitable one giving symmetrical well resolved peaks for FLV and its degradation products within a short analysis time. On the other hand, FLV was strongly retained on the C_8 column so that no peaks appeared in the chromatogram even after 40 min.

This can be explained by the high lipophilicity of FLV since it has Log P *(octanol/water)* = 4.9, [15] so, it tends to bind strongly with this non-polar stationary phase. On the other hand, the cyanopropyl column yielded asymmetrical broad peak of FLV overlapped with its degradation product (DP1).

Choice of appropriate detection wavelength

The absorption spectrum of FLV exhibits three maxima at 240, 290 and 325 nm. A wavelength of 325 nm was selected as the optimum detection wavelength throughout this study.

Effect of pH of the mobile phase

FLV is a basic compound with pK_a value of 7.3 [15] corresponding to the piperidine nitrogen. Over the normal working pH range for best life times for most reversed phase columns (2.0-7.0), the cationic species of FLV is predominant and it is expected that there will be no significant changes in its retention behavior. This was confirmed by experimental study using mobile phases of pH values over the normal working range. The retention of FLV was scarcely affected by changes in the pH of the mobile phase (Fig. 2A). pH 2.5 was selected as the optimum pH value in the study. At this pH, the ionization of silanol groups of the stationary phase is reduced thus, minimizing its interaction with FLV, which is present as a cationic species. Moreover, at pH 2.5 the column efficiency was maximal as revealed by the highest number of theoretical plates.

Effect of SDS concentration

SDS concentration in the mobile phase was studied over the range of 0.10-0.15 M. It was observed that, an increase in SDS molar concentration produced a corresponding decrease in the retention factor (k') of FLV (Fig.

2B). A mobile phase containing 0.15 M SDS was finally selected as the optimum one taking in consideration the total analysis time, peak symmetry and column efficiency.

Effect of type of organic modifier

The presence of organic modifiers such as short chain alcohols in micellar mobile phase is usual because their addition improves the retention and the peak efficiency. [13, 14] In addition, the presence of an organic modifier in the micellar mobile phase also alters the retention mechanism by shifting equilibrium of the solutes from the stationary phase, and from the micelle toward the bulk aqueous phase. This leads to a reduction in the retention factors. [13, 14]

To select the optimum organic modifier for separation of FLV from its degradation products, four mobile phases containing *n*-propanol (15% v/v), methanol (18% v/v), pentanol (7% v/v) and tetrahydrofuran (15% v/v) were tested. The results obtained indicated that *n*-propanol is the best organic modifier since it gave symmetrical peaks with best resolution. On the other hand, pentanol caused co-elution of FLV with its degradation product (DP1). The use of methanol or tetrahydrofuran as organic modifiers caused broadening of FLV peak.

Effect of concentration of organic modifier

To study the effect of the concentration of *n*-propanol in the mobile phase on the separation of FLV and its degradation products, its percentage in the mobile phase was varied over the range of 10-15% v/v. The retention of FLV decreased with the increase in *n*-propanol percentage (Fig. 2C). A concentration of 15% v/v *n*-propanol was chosen as the optimal concentration, where it provided a good combination of peak symmetry, sharpness, resolution factor and short analysis time.

After this experimental study, when maximum resolution-minimum analysis time criteria was applied, the mobile phase selected as being optimal was 0.15 M SDS-15% *n*-propanol-0.3%TEA-0.02 M H_3PO_4 at pH 2.5 with UV-detection at 325 nm. TEA was used as a component in the mobile phase to protect the silanol groups of the stationary phase and increase the peak efficiency of FLV which is a basic compound. [15] Fig. 3A illustrates a representative chromatogram for FLV in pure drug substance under the optimum chromatographic conditions.

Method Validation

Following ICH guidelines Q2 (R1) for validation of analytical methods, [16] the proposed method was validated to demonstrate its linearity, range, limit of quantification (LOQ), limit of detection (LOD), accuracy, specificity, precision, stability of sample solution and mobile phase and system suitability test parameters.

Linearity and range

Calibration plot for FLV was evaluated and checked by analyzing standard solutions at 6 concentration levels, ranging from 2.0 to 40.0µg mL^{-1}. The validity of the method was proven by statistical evaluation of the regression line. [17] The data provide conclusive evidence of linearity between concentrations and peak areas. The fairly small values of the standard deviation of the residuals ($S_{y/x}$), slope (S_b) and intercept (S_a), and the % relative error indicate low scattering of the calibration points around the regression line (Table 1).

Limit of quantification (LOQ) and limit of detection (LOD)

According to ICH recommendation Q2 (R1), [16] the approach based on the standard deviation of intercept of the regression line and the slope was used for calculating LOQ and LOD adopting the following equations:

$LOQ = 10S_a/b$

$LOD = 3.3S_a/b$

Where: S_a is the standard deviation of the intercept of regression line and b is its slope.

The obtained results are abridged in Table 1.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. [16] To test the validity of the proposed method, it was applied for the determination of pure samples of FLV over the concentration range of 2.0-40.0 μg mL^{-1}. The results obtained were in good agreement with those obtained using the comparison HPLC method. [7] Using the Student's *t*-test and the variance ratio *F*-test [17] revealed no significant difference between the performance of the two methods regarding accuracy and precision, respectively (Table 2).

Precision

Precision of the method was evaluated by intra-day precision (repeatability) and inter-day precision (intermediate precision) experiments. The intra-day precision was estimated from three consecutive injections for three concentration levels over the whole linearity range. For evaluation of inter-day precision, three standard solutions of FLV covering the entire linearity range were analyzed over three successive days. All data from precision study are summarized in Table 3. The obtained results indicate the

high precision of the proposed method as revealed by small values of standard deviation and relative standard deviation. [16]

Specificity

The specificity of the proposed method was tested by assaying FLV in the presence of its degradation products formed during exposure to different accelerated degradation conditions. Results of specificity study revealed that FLV and its degradants were well-separated and no peaks interfering with the elution of FLV was observed, thus demonstrating that the proposed method is specific and stability-indicating. Moreover, no interferences from common tablet excipients with the peaks of interest were observed in the analysis of a placebo formulation, confirming the specificity of the method.

Robustness

Robustness of the proposed method was evaluated by studying the effect of small deliberate changes of the chromatographic conditions on resolution and peak area. The method was found to be robust to small changes in experimental conditions including SDS concentration (0.15 ± 0.001 M), proportion of *n*-propanol (15 ± 0.5 %), and pH of the mobile phase (2.5 ± 0.2).

System suitability test

Resolution (R_s), number of theoretical plates (N), selectivity (α) and retention factor (k') were measured as the criteria for system suitability testing according to ICH guidelines Q2 (R1) [16] as shown in Table 4.

Applications

Application of the proposed method to investigate the degradation behavior of FLV according to ICH recommendations

The developed MLC method was applied to study the degradation behavior of FLV under different stress conditions such as alkaline, acidic, neutral, oxidative and photolytic conditions according to ICH guidelines. [11, 12] A summary of this study is described below.

- *Degradation behavior under hydrolytic conditions (acidic, alkaline and neutral conditions)*

FLV is an ester-type drug that is expected to be susceptible to hydrolysis. So, it was necessary to study the degradation behavior of FLV under different hydrolytic conditions including acidic, alkaline and neutral hydrolysis.

Under acidic conditions (1.0 M HCl, 100 ºC, 30 min), about 17% of the drug was degraded and two degradation products, DP1 and DP2, appeared in the chromatogram at 5.9 and 4.9 min, respectively (Fig. 4A). Meanwhile, in case of neutral hydrolysis (water, 80 ºC, 30 min), only 11% of the parent drug was degraded with the appearance of DP1 and DP2 also (Fig. 4B). The degradation behavior of FLV in water may be attributed to presence of HCl as a part of drug substance itself. [3]

In alkaline medium, the hydrolysis occurs faster. The alkaline hydrolysis was investigated in 0.1 M NaOH at room temperature for 10 min, where complete hydrolysis of FLV occurred with the appearance of DP1 and DP2 (Fig. 4C).

Being an ester, the degradation of FLV under hydrolytic conditions is expected to proceed *via* hydrolysis of the ester linkage yielding the corresponding acid form, 3-methylflavone-8-carboxylic acid (DP1). [7] In

addition, FLV belongs to chromone compounds which are reported to undergo pyrone ring opening with the formation of a 2-hydroxyphenyl alkyl ketone when heated with alkali or acid. [18] Thus, it can be inferred that FLV acid form (DP1) undergoes pyrone ring opening yielding DP2 as illustrated in Scheme 1. The degradation product of higher polarity (DP2) eluted first at 4.9 min where that of lower polarity (DP1) eluted later at 5.9 min.

- *Degradation behavior under oxidative conditions with kinetic investigation*

The oxidative degradation of FLV was investigated by heating with H_2O_2. Preliminary studies revealed that FLV is susceptible to oxidative degradation, where, considerable degradation was observed with the formation of DP1 and DP2 (Fig. 5A). Consequently, the kinetics of oxidative degradation was explored at different temperature settings (60-80 °C) for increasing time intervals (10-40 min) (Fig. 5B). The oxidative degradation of FLV was found to follow first order degradation kinetics. The apparent first order reaction rate constants and the half-life times were calculated and the results are presented in Table 5. By plotting log K_{obs} values versus 1/T, Arrhenius plot was obtained [19] (Fig. 5C). Arrhenius equation [19] was found to be:

$\text{Log } K = 0.696 - 1079/T$

Where: K is the reaction rate constant (min^{-1}) and T is the absolute temperature (°Kelvin).

Hydrogen peroxide is known to react with tertiary amines to form tertiary amine oxides. [20] It is expected that oxidation of FLV occurs through the formation of N-oxide form. [3] Under the effect of heating; the N-oxide form will undergo instant hydrolysis yielding DP1 which will further decomposed to DP2 as previously illustrated (Scheme 1).

The activation energy (E_a) of the oxidative degradation of FLV was calculated and was found to be 4.94 K. Cal. mol^{-1}. This value agreed with the

results of El-Gindy *et al* [7] who studied the kinetics of acidic hydrolysis of FLV and calculate the activation energy of this reaction to be 4.343 K. Cal. mol^{-1}.

- *Photolytic degradation in different solvent systems*

FLV belongs to flavone derivatives which are known to be photosensitive and their photosensitivity depends on the characteristics of the reaction environment and is influenced by the medium polarity. [21] This fact makes it essential to investigate the photostability of FLV in different solvents under UV-irradiation. FLV solutions (400 μg mL^{-1}), in methanol, water and methanol: water mixture (1:1 v/v), were exposed to UV irradiation at 254 nm for 3 hrs. An interesting photodegradation pattern was observed. Irradiation of FLV methanolic solution resulted in photodegradation of FLV with the appearance of DP1 at 5.9 min, while aqueous solution of FLV underwent photodegradation but with the appearance of DP2 at 4.9 min. On the other hand, irradiation of FLV solution in methanol: water system (1:1 v/v) resulted in photodegradaion with the appearance of both DP1 and DP2. The obtained results agreed with published literature about flavone compounds photoreactivity [21] which approves that the medium polarity affects the photodegradation mechanism of flavone molecules on UV exposure. The results of photodegradation studies revealed the photosensitivity of FLV. These results are in accordance with precaution stated by the British Pharmacopoeia [2] which recommended protection of FLV from light.

The results of the photodegradation study of FLV in different solvents are represented in Fig. 6A-6C.

- *Stability of FLV standard solution during normal analysis time and under storage conditions*

The normal time of analysis in a quality-control laboratory is around 6 hrs, so it is essential to evaluate the stability of standard solution over this

period to obtain reliable results. A standard methanolic solution of FLV was prepared and kept at the laboratory temperature exposed to artificial day light for 6 hrs. The response of this solution was compared with that of a freshly prepared standard solution. The obtained results indicated that the drug degraded (8%) yielding a degradation product (DP1) at 5.9 min (Fig. 7A).

In addition, the stability of methanolic FLV standard solution stored in the refrigerator at 4 °C was checked. Complete degradation of FLV was observed after storage under these conditions for 7 days with appearance of DP1 at 5.9 min (Fig. 7B). Consequently, it is recommended to freshly prepare the drug every working day, protect it from light and check its stability during quality control analysis time.

A summary of the results of stability studies of FLV is presented in Table 6.

Pharmaceutical applications

The proposed MLC method was applied for the determination of FLV in Genurin S.F® 200 tablets and the results are presented in Table 2. The good percentage recoveries with small SD value confirm the suitability of the proposed method for the routine determination of this compound in commercial tablets. As can be seen, the nominal tablet content agreed well with those declared by the manufacturer. Statistical analysis of the results obtained by the proposed method and those given by the comparison method was performed using Student's *t*-test and variance ratio *F*-test. [17] As illustrated in Table 2, the calculated *t*- and *F*- values did not exceed the theoretical ones, indicating no significant difference in the performance of the compared methods regarding accuracy and precision, respectively. Fig. 3B represents the chromatogram obtained for Genurin S.F® 200 tablets analyzed under the optimum chromatographic conditions. The obtained results indicate the stability of FLV tablet formulation under normal storage conditions

recommended by the manufacturer, where, no degradation products were detected in the obtained chromatograms.

Conclusion

A rapid, reliable, specific and accurate stability-indicating MLC method was developed and validated for the assay and stability study of FLV. Specificity of the method in relation to degradation products was entirely proved for FLV assay. Nevertheless, this paper is the first report that investigates the oxidative and photodegradation processes of this drug. The method is suitable for the quality control of commercial FLV tablets. MLC analysis meets the requirements of *"green chemistry"* conception by using environment-friendly reagents. Micellar mobile phase is less toxic and non-flammable and has lower environmental impact compared to conventional hydro-organic HPLC methods.

Authors' contributions

Rania N. El-Shaheny planned the study, carried out the experimental work, performed statistical analysis of the data and wrote the manuscript, Nahed El-Enany suggested the idea of the study and Fathalla Belal supervised the work. All authors approved the final article.

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Table 1 Analytical parameters for the determination of FLV by the proposed method

Parameter	Results
Concentration range (μg mL^{-1})	2.0-40.0
Limit of detection (LOD) (μg mL^{-1})	0.40
Limit of quantification (LOQ) (μg mL^{-1})	1.20
Correlation coefficient (r)	0.9998
Slope	4.52×10^4
Intercept	1.08×10^4
Standard deviation of the residuals ($S_{y/x}$)	1.19×10^4
Standard deviation of the intercept (S_a)	5.42×10^3
Standard deviation of the slope (S_b)	3.52×10^2
% RSD	1.25
% Error (% RSD/√n)	0.51

Table 2 Application of the proposed and comparison methods to the determination of FLV in pure form and tablets.

Matrix	Proposed method			Comparison method (7)
	Conc. taken ($\mu g\ mL^{-1}$)	Conc. found ($\mu g\ mL^{-1}$)	% Found[a]	% Found[a]
Pure form	2.0	1.973	98.65	101.49
	4.0	3.945	98.63	98.01
	10.0	9.883	98.83	100.33
	20.0	20.073	100.37	
	30.0	30.506	101.69	
	40.0	39.620	99.05	
$\bar{X}$ ± SD			99.53 ± 1.24	99.94 ± 1.77
t			0.407 (2.365)*	
F			2.045 (5.786)*	
Genurin S.F.®	10.0	9.840	98.40	101.42
tablets (200 mg	20.0	20.241	101.21	98.11
FLV/tablet)	40.0	39.920	99.80	100.32
X ± SD			99.80 ± 1.41	99.95 ± 1.69
t			0.116 (2.776)*	
F			1.440 (19.00)*	

[a] Each result is the average of three separate determinations.

* Values between parenthesis are the tabulated *t* and *F* values at *P*=0.05 (17).

Table 3 Precision data of the proposed method for determination of FLV in pure form.

Conc. (μg mL^{-1})	% Found ± SD	% RSD	% Error
	Intra-day precision		
4.0	99.13 ± 1.02	1.03	0.59
10.0	97.71±1.40	1.40	0.80
40.0	99.98±1.04	1.04	0.60
	Inter-day precision		
4.0	101.20±1.25	1.24	0.71
10.0	99.98 ± 1.10	1.10	0.64
40.0	98.60±0.90	0.90	0.50

Table 4 Final system suitability test parameters for the proposed method

Parameter	FLV	DP2	DP1
No of theoretical plates, N	615	1011	850
Capacity factor, k'	1.73	0.91	1.33
Selectivity factor, α		1.90	1.30
Resolution, R_s		2.41	1.04

Table 5 Reaction rate constants and half-life times of FLV in H_2O_2 solution (30% w/v) at different temperature settings

Temperature (°C)	K (min^{-1})	$t_{½}$ (min)
60	2.8×10^{-3}	247
70	3.9×10^{-3}	178
80	4.6×10^{-3}	151

K: reaction rate constant (min^{-1}), $t_{½}$: half-life time (min).

Table 6 Results of the degradation study of FLV

Stress condition	t_R of degradation product(s) (min)	% Assay of FLV
0.1M NaOH, 10 min, RT	4.9, 5.9	0
1.0 M HCl, 100 ºC, 30 min	4.9, 5.9	83
30% w/v H_2O_2, 80 ºC, 30 min	4.9, 5.9	86
Water, 80 ºC, 30 min	4.9, 5.9	89
Methanol, 7 days, 4 ºC	5.9	0
UV light, methanol, 3 hrs	5.9	76
UV light, water, 3 hrs	4.9	90
UV light, methanol: water (1:1 v/v), 3 hrs	4.9, 5.9	82
Artificial day light, methanol, RT, 5 hrs	5.9	92

t_R: Retention time (min), RT: Room temperature

List of figures:

Fig. 1 Chemical structure of Flavoxate hydrochloride (FLV).

Fig. 2 Effect of: (A) pH, (B) SDS conc. [M] and (C) *n*-Propanol conc. (%) on the retention factor of FLV.

Fig. 3 Representative chromatograms showing:

(A) FLV (40µg mL^{-1}) standard solution.

(B) FLV (40µg mL^{-1}) in Genurin SF® tablets.

Fig. 4 Representative chromatograms showing FLV (40µg mL^{-1}) after exposure to different hydrolytic conditions:

(A) Acidic condition (1.0 M HCl, 80 ºC, 30 min)

(B) Neutral condition (water, 80 ºC, 30 min).

(C) Alkaline condition (0.1M NaOH, 10 min, RT).

Fig. 5:

(A) Representative chromatograms showing FLV (40µg mL^{-1}) after exposure to oxidative degradation (30% w/v H_2O_2, 80 ºC, 30 min).

(B) 3D plot showing the effect of heating time with H_2O_2 (30% w/v) on FLV (40µg mL^{-1}) at different temperature settings (*a* is the initial drug concentration and *a-x* is the remaining concentration of the drug).

(C) Arrhenius plot for the oxidative degradation of FLV (40µg mL^{-1}) with H_2O_2 (30% w/v).

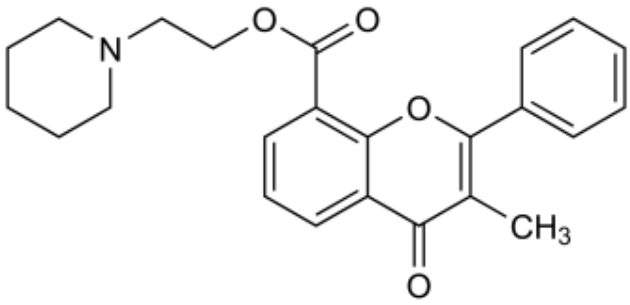
Fig. 6 Representative chromatograms showing FLV (40µg mL^{-1}) after exposure to UV irradiation at 254 nm for 3 hrs in different solvent systems:

(A) Methanol, (B) Methanol: water (1:1 v/v) and (C) Water.

Fig. 7 Representative chromatograms showing:

(A) FLV (40 µg mL^{-1}) after storage for 5 hr at room temperature.

(B) FLV (40 µg mL^{-1}) after storage for 7 days at 4 °C.



. HCl

Fig. 1

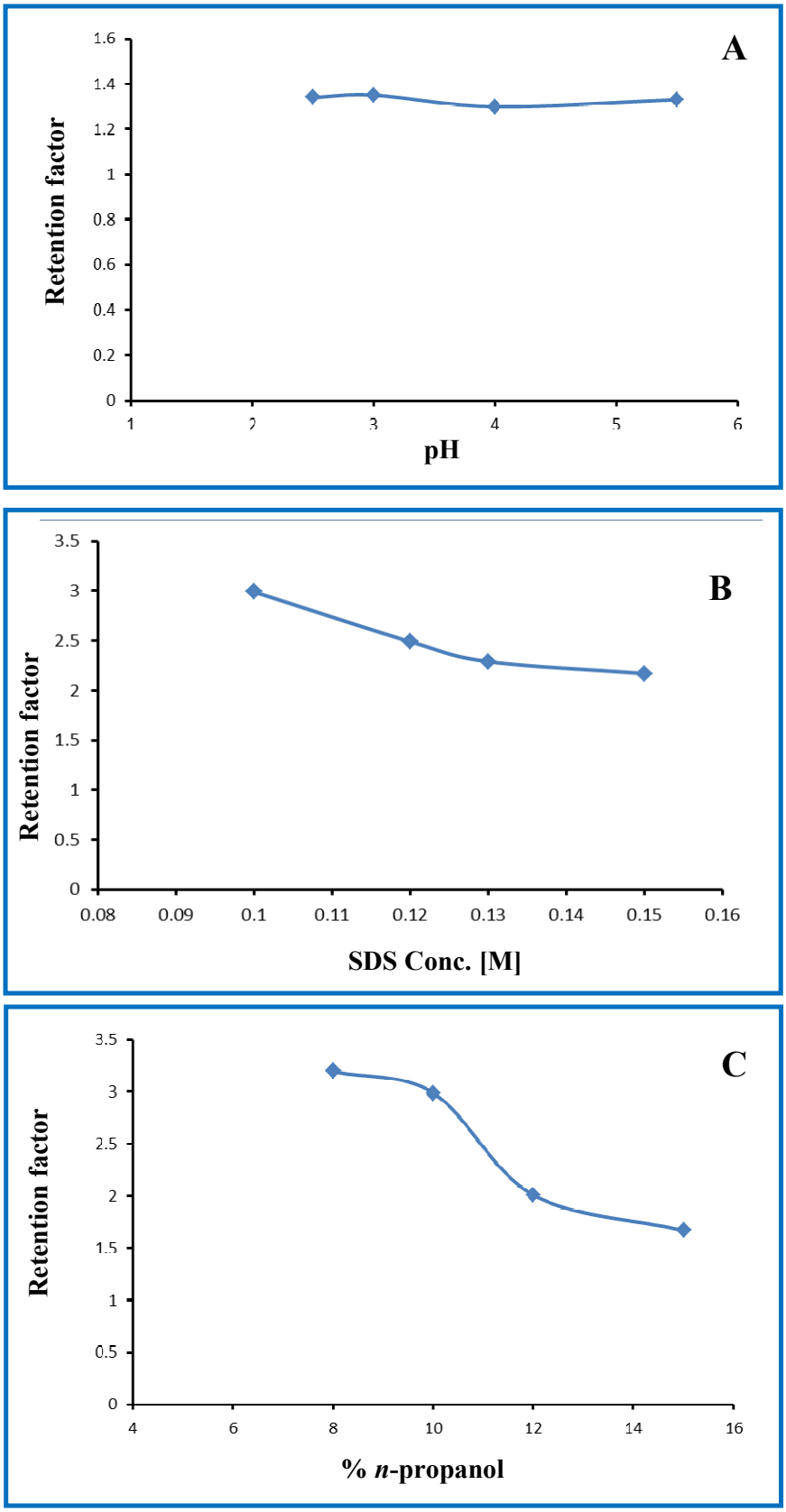
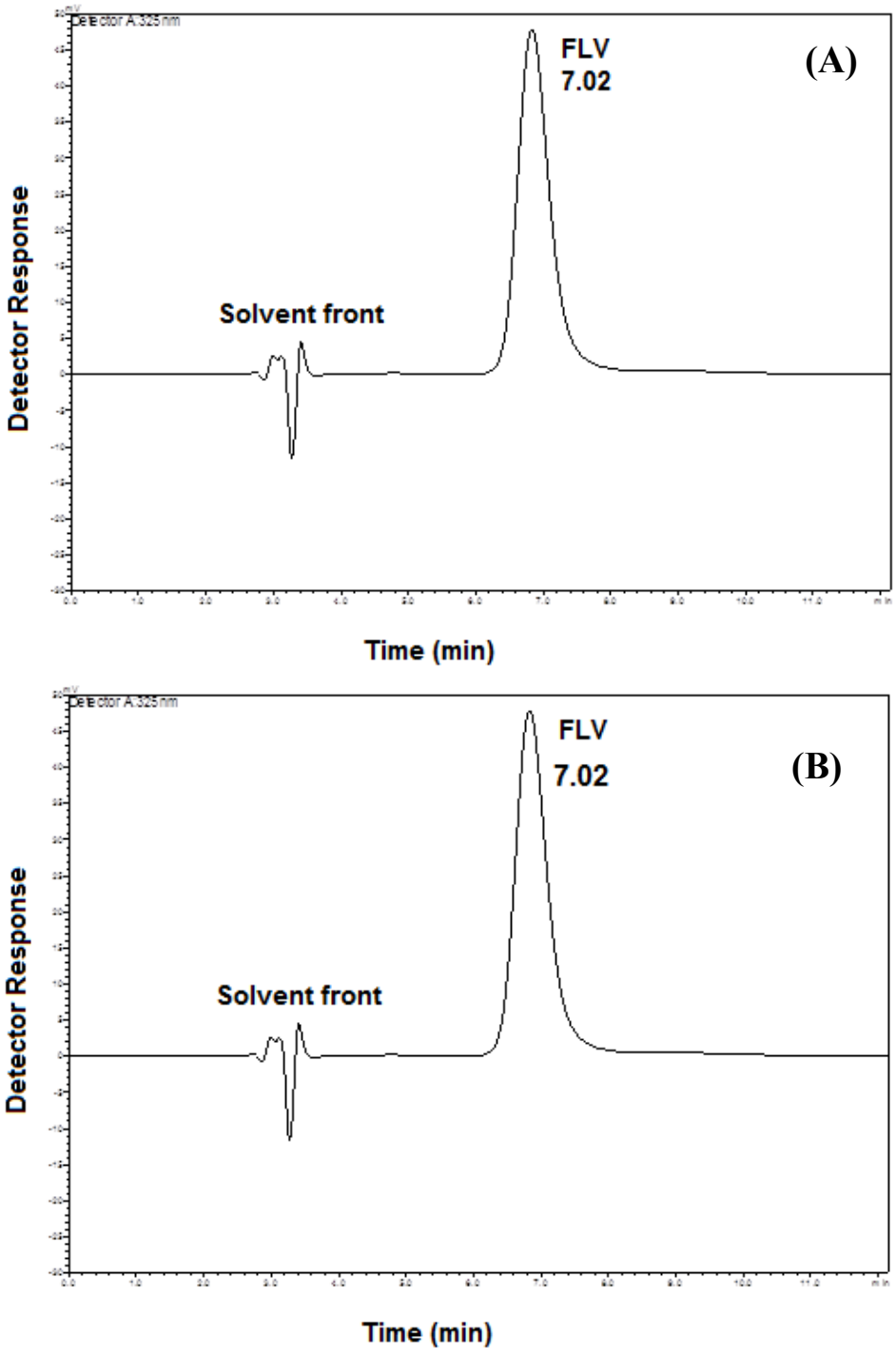


Fig. 2



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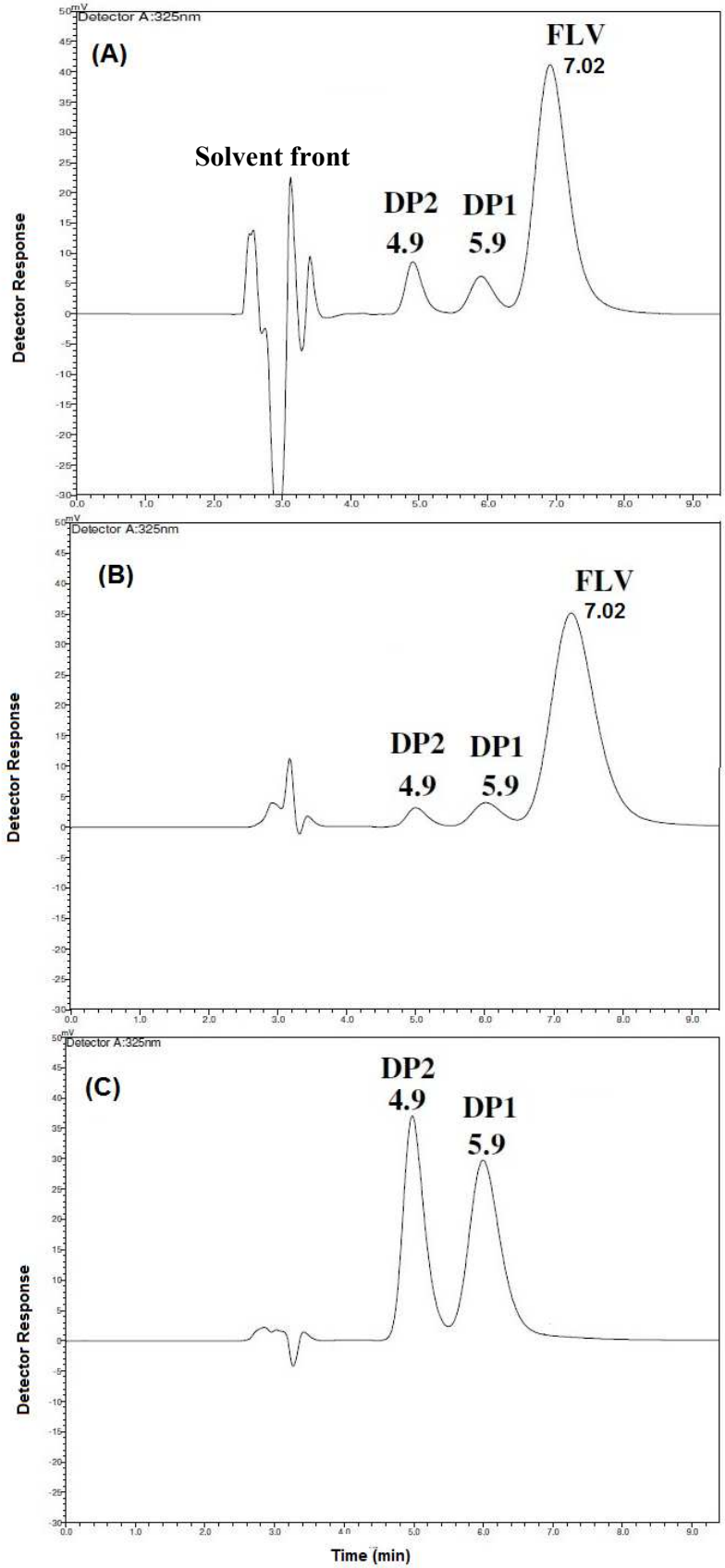
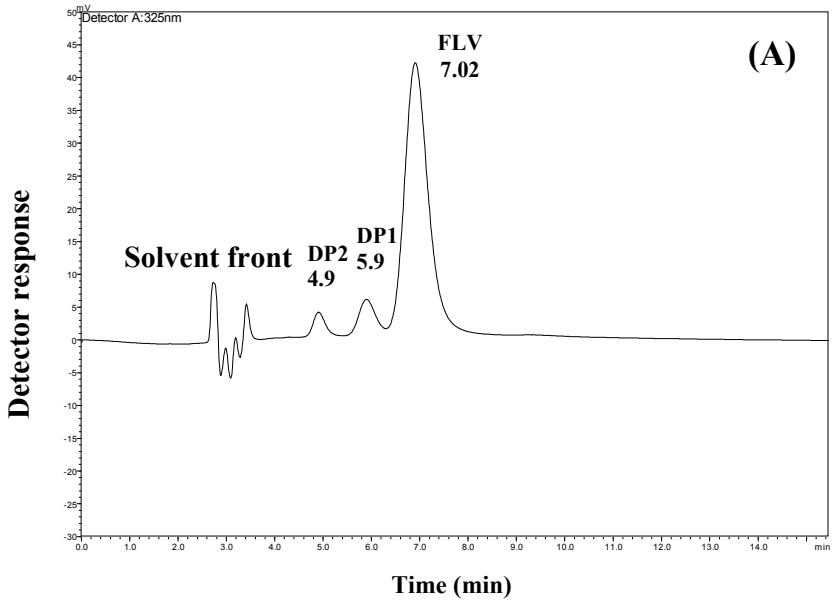
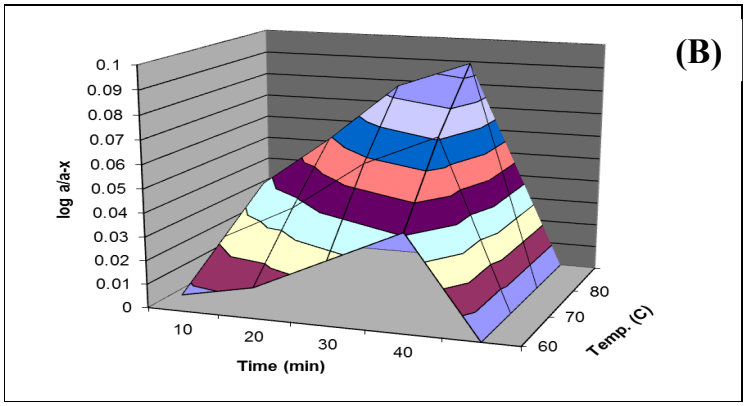


Fig. 4





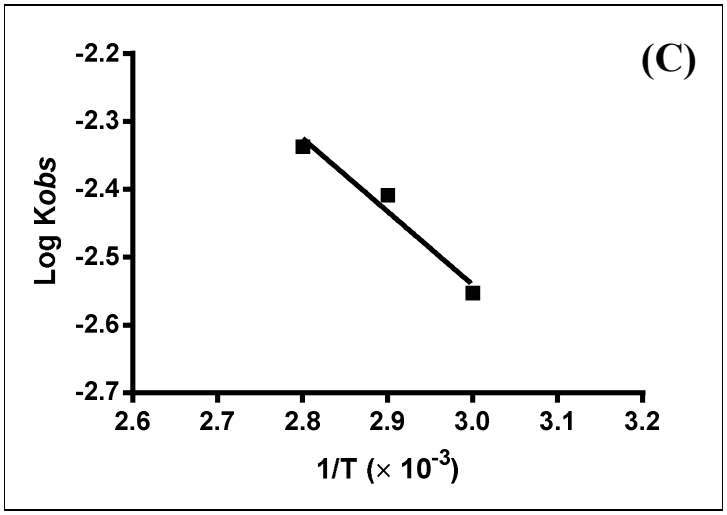


Fig. 5

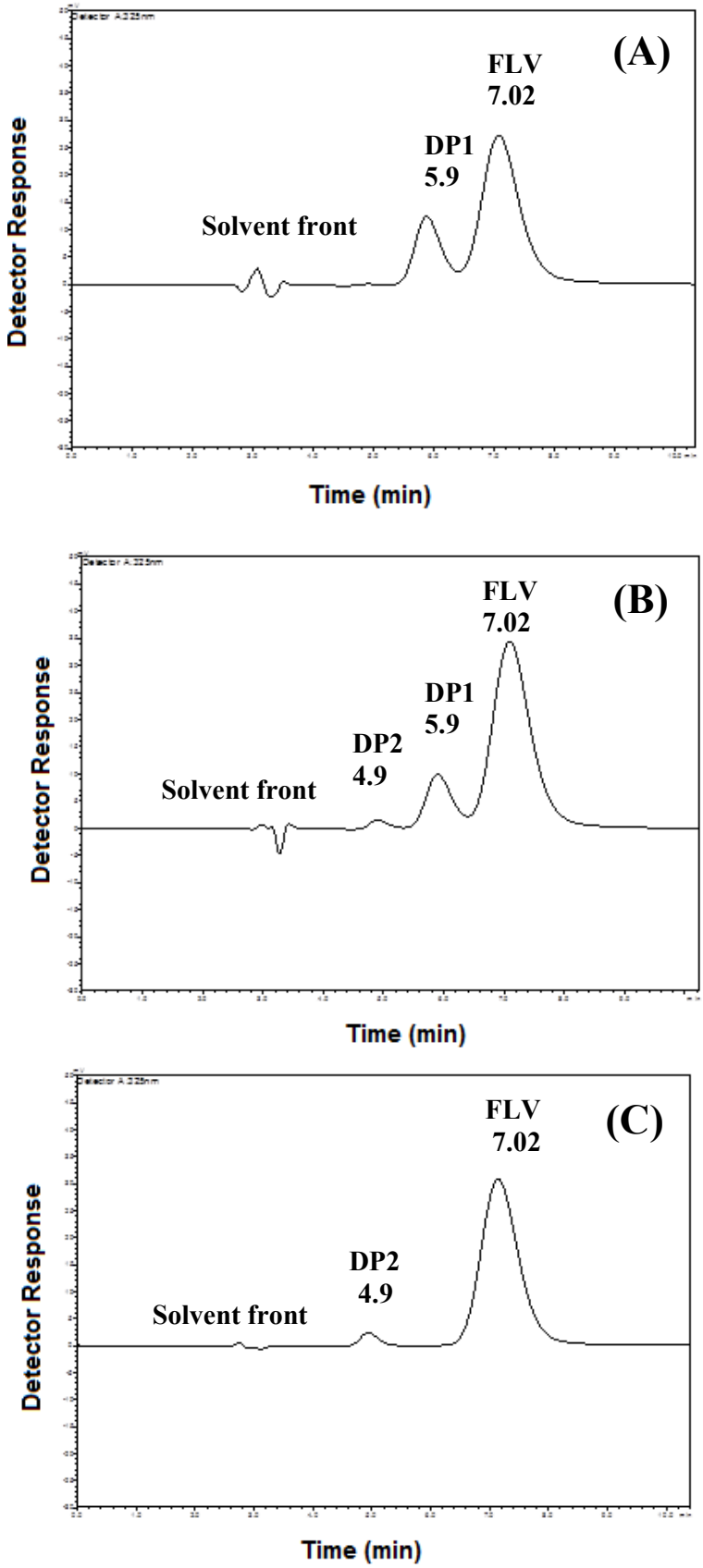


Fig. 6

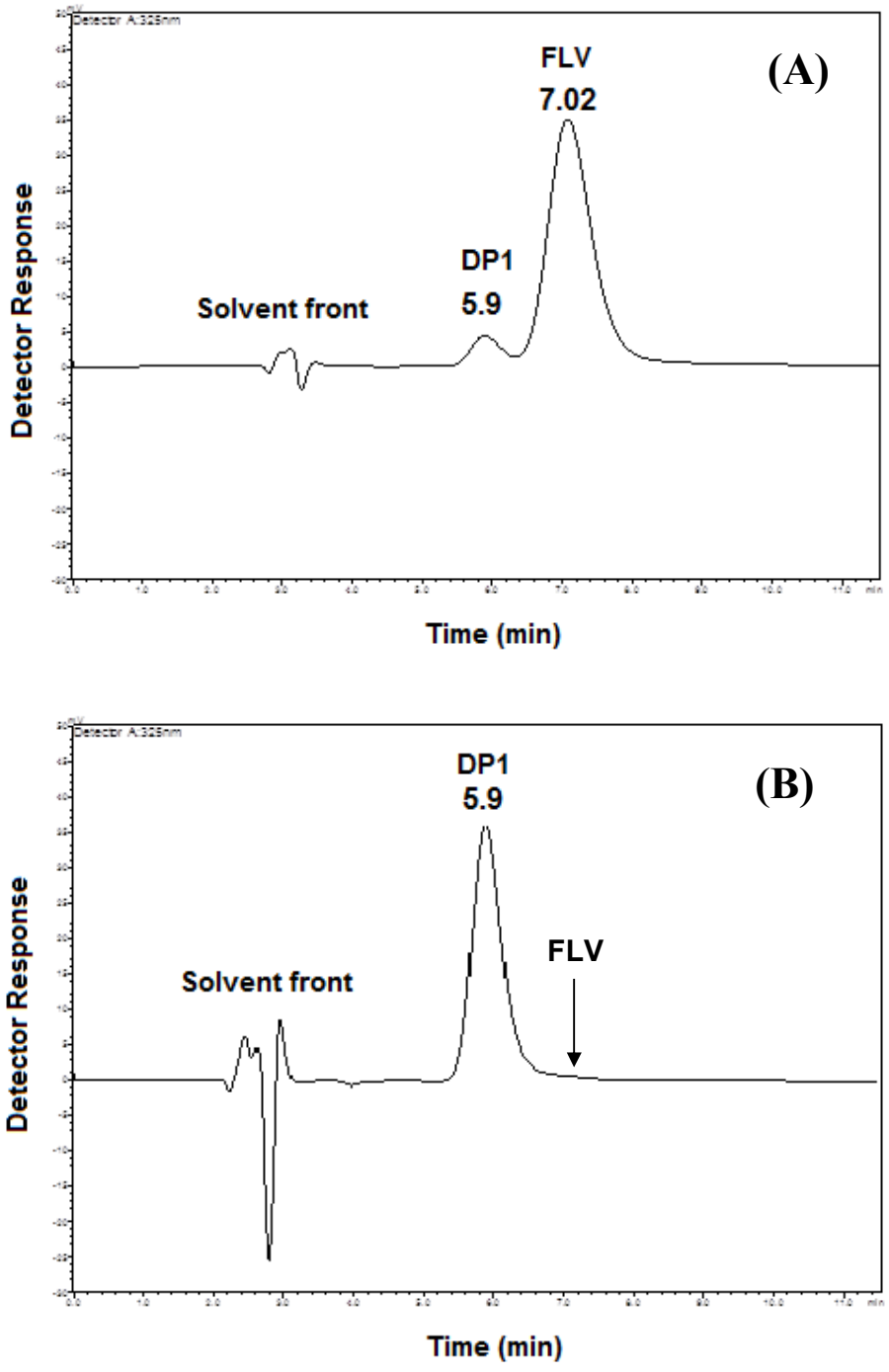
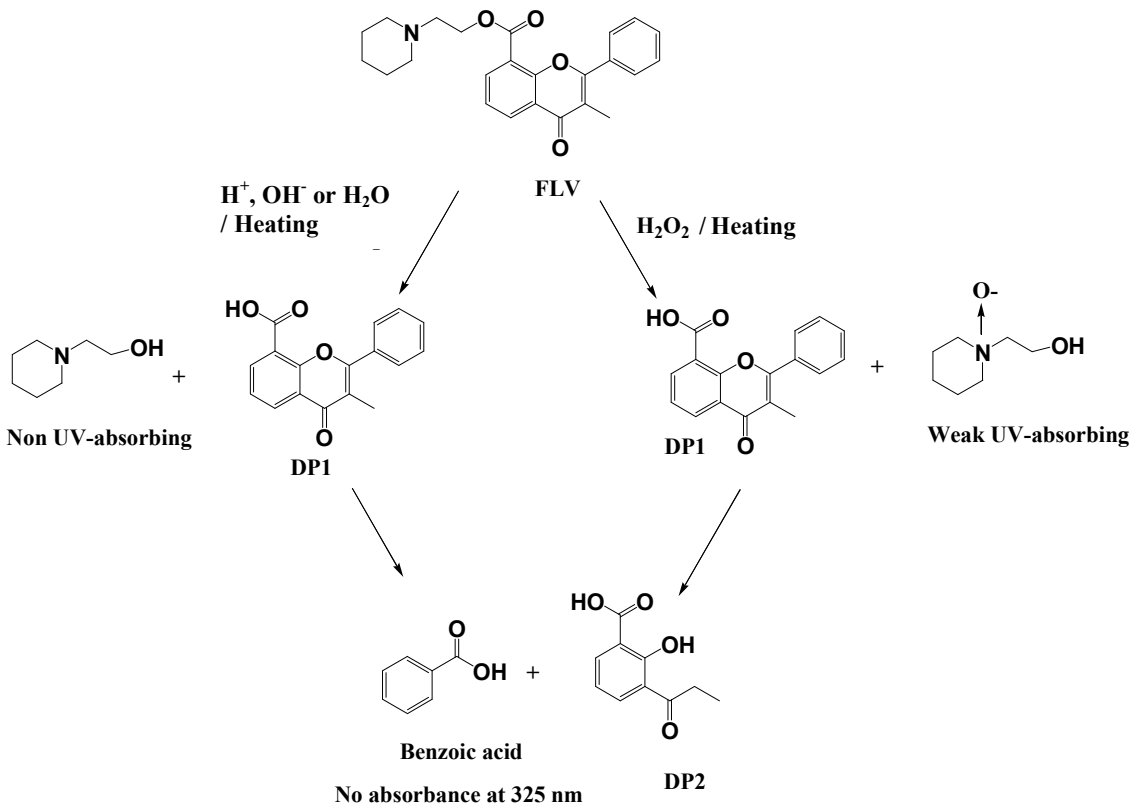


Fig. 7



Scheme 1 Suggested degradation pathways of FLV