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Micellar Liquid Chromatographic Determination of Idrocilamide in Dosage Form and Biological Fluids: Application to Stability Study

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Abstract

A simple, stability-indicating, reversed phase high perfomance liquid chromatographic method was developed and validated for the determination of idrocilamide in the presence of its degradation products .The separation was conducted using A Hibar C_{18} (150 × 4.6 mm i.d) stainless steel column at ambient temperature with UV detection at 277 nm . Micellar mobile phase consisted of 0.1 M sodium dodecyl sulphate, 10% n-propanol, 0.3 % triethylamine in 0.02 M phosphoric acid (pH 6) was used and pumped at a flow rate of 1 mL/min .The calibration curve was rectilinear over the concentration range of 1-10 µg/mL with a detection limit of 0.1 µg /mL and quantification limit of 0.3 µg/mL .The proposed method was successfully applied to the analysis of idrocilamide in commercial cream with mean % recovery of 100.74 ± 0.93. The method was extended to the *in-vitro* determination of idrocilamide in spiked human plasma and urine samples with mean % recoveries of 99.93 ± 0.31 and 100.1 ± 0.26 respectively Moreover, the method was utilized to investigate the kinetics of both acid and alkaline induced degradation of the drug . The apparent first –order rate constant, half life time and activation energies of the degradation reactions were calculated

Keywords: Human plasma; Urine samples; Idrocilamide; Mobile phase

Introduction

Idrocilamide is N-(2-hydroxyethyl)cinmamamide [1]. It is used as central muscle relaxant when given by mouth or intramuscular. It is reported to have local muscle relaxant and anti- inflammatory effects; and is now mainly used topically [2]. The literature is poor in the reported methods for the analysis of idrocilamide. Only two HPLC methods were reported for its determination, one using 60 % methanol [3] and the other 65 % methanol [4] as mobile phases. Two Stability –indicating methods were also developed for its determination [5,6]. HPTLC and HPLC methods were introduced for determination of idrocilamide in presence of its degradation products [5]. The second method is based on the use of first derivative spectrophotometry for the analysis in tablets. This fact motivated us to develop a simple and reliable method for its determination in dosage form and biological fluids. Micellar liquid chromatography (MLC) has proved to be a useful technique in the determination of diverse groups of compounds in several matrices including food samples. MLC allows complex matrices to be analysed without the aid of extraction and with direct injection of the samples. Micelles tend to bind proteins competitively, thereby releasing protein-bound drugs and proteins, rather than precipitating into the column. Proteins compounds are solubilized and washed harmlessly away, eluting with the solvent front. MLC generate less amount of toxic waste in comparison to aqueous-organic solvents, so that they are less toxic, non-flammable, biodegradable and relatively inexpensive [7]. Micelles provide hydrophobic and electrostatic (for ionic surfactants) sites of interaction. In the micelles, three sites of solubilisation can be identified: the core (hydrophobic), the surface (hydrophilic) and the palisade layer (the region between the surfactant head groups and the core). Solutes associated to micelles experience a microenvironment that is different from that of bulk solvent. This is reflected by micelle-induced perturbations in solute physicochemical properties, including changes in solubility, acidity, photophysical properties, and reaction rates [8]. It has also other advantages like using small amounts of organic modifier which is retained in the micellar solution of SDS, thus reducing the risk of evaporation and make the micellar mobile phase more stable. Also, the capability of simultaneous separation of hydrophobic and hydrophilic analytes in the same run without a gradient elution [9]. Unfortunately, some drawbacks were reported concerning the chromtographic efficiency and weak elution strength of pure micellar solution [10,11]. Many articles has been reported for determination of drugs in pharmaceutical preparations and biological fluids [12-14]. In our laboratory, MLC proved to be a useful technique in the quality control of several drugs in pharmaceutical preparations using hybrid micellar mobile phase containing SDS and organic modifier [15-17]. The proposed method is superior over the reported methods in the ease of biological fluids analysis where direct injection of plasma and urine is investigated.

Experimental

Reagents

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

- Orhtophosphoric acid (Prolabo, Paris, France).
- Methanol (Hipersolv, Merck).
- Sodium dodecyl sulphate (Park Scientific limited, Northompthon, UK) 0.1 M aquous solution was prepared.
- n- Propanol, Ethanol and Triethylamine (Riedel- ole Haen, Sleeze, Germany).

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- n- Butanol (Honi limited, London, UK).

Materials

1) Idrocilamide was kindly supplied by Minapharm Pharmaceuticals,10th of Ramadan city, Egypt. The purity of the drug was established by applying the comparison method [3] and was found to be 99.88±0.41

2) Srilane® cream 5 % (Minapharm Pharmaceuticals) was obtained from the local market (Batch number B5468).

3) Plasma was obtained from Mansoura University Hospital and was kept frozen untill use after gentle thawing.

4) Urine samples were obtained from healthy volunteer (male 27 years old).

Instrument

- Separation was performed using a Perkin Elmer^{tm} Series 200 Chromatograph equipped with a Rheodyne injector valve with 20 µL loop and a UV/VIS detector set at 277 nm Total Chrom Workstation was applied for data collection and processing (MA, USA)

- A Shimadzu UV 1601 PC Spectrophotometer equipped with a pair of 1cm matched quartz cells. Recording range: 0-2, wavelength 277 nm ; factor : 1; number of cells : 1; cycle time: 0.1 min.

Column and mobile phase

EC 150/406 Hibar 100-5 C_{18} column was used. The components of the micellar mobile phase was 0.1 M sodium dodecyl sulphate (SDS), 10% n-propanol and 0.3 % triethyl amine in 0.02 M phosphoric acid (pH 6). The mixture was then shaken using an ultrasonic bath for 30 min. The resulting transparent mobile phase was filtered through a 0.45 µm membrane filter (Millipore, Ireland). The micellar mobile phase was freshly prepared. The column hold up value was the first deviation of the base line obtained.

Standard solutions

Stock solution of idrocilamide was prepared by dissolving 10.0 mg of the drug in few mL of methanol then diluted with distilled water to 25.0 mL in a 25 mL volumetric flask. The stock solution

was further diluted with the mobile phase to obtain the working concentration range (1-10 µg/mL) . The stock solution was found to be stable for at least one week when kept in the refrigerator .

General procedures

Construction of calibration graph: Aliquots of the working solution were transferred into a series of 10 ml volumetric flasks and diluted with the mobile phase to the mark so that the concentration of these solutions was in range of 1-10 µg/ml, 20 µL aliquots were injected (triplicate) and eluted with the mobile phase. A plot of the peak area versus concentration of the drug was constructed to obtain the standard calibration curve .Alternatively, the regression equation was derived.

Acidic and Alkaline degradation of idrocilamide. Aliquot volumes of idrocilamide standard solution were mixed with 5 mL of either 2 M NaOH or 1 M HCl, the solutions were heated at different temperature settings 60,70,80,90,100°C using a thermostatically controlled water bath for different time intervals 5,15,25,35,45 minutes then allowed to cool. The solutions were neutralized to pH 7 using 2 M HCl or 1 M NaOH respectively. Then aliquot volumes of these solutions were transferred to 10 mL volumetric flasks so that the final concentration was within the working concentration range and treated as described under "Construction of the calibration graph". Complete degradation was tested by the disappearance of HPLC peak of the parent drug.

Analysis of Idrocilamide in cream: The contents of five tubes were mixed and an amount of the cream equivalent to 0.05g of idrocilamide was accurately weighed and extracted with methanol, filtered and diluted with the same solvent to the mark in a 100 mL volumetric flask. This solution was serially diluted with the mobile phase to get the working concentration range in a series of 10 mL volumetric flasks. 20µL were injected (triplicate) and eluted with the mobile phase. The nominal content was obtained either from the calibration graph or using the regression equation.

Analysis of idrocilamide in spiked plasma and urine

1.0 mL aliquots of sample plasma or urine were transferred into a series of centrifuge tubes, spiked with increasing concentrations of idrocilamide to obtain the working concentration 1-10 µg/mL. 20 µL were injected (triplicate) directly without extraction and eluted with the micellar mobile phase. The nominal concentration was obtained from the corresponding regression equation.

Results and Discussion

The acid and alkaline degradation of idrocilamide was found to give one and the same degradation product. In both cases the amide group being hydrolysed yielding the same reaction products cinnamic acid and hydroxyl amine. The latter is non- UV absorbing, therefore in both cases, only one peak of the degradation product appears. The peaks of the drug and its degradation product are well separated from each other (resolution factor Rs=2.4) and the retention times were 1.5 and 3.2 for cinnamic acid (CA) and idrocilamide respectively.

UV Detection

The UV absorption spectrum of idrocilamide was scanned and the λ_{max} 277 was selected for detection of peaks (Figure 2 shows the spectra of idrocilamide before and after degradation).

Study of the Experimental parameters

The experimental parameters affecting the chromatographic separation of the drug and cinnamic acid (CA) including mobile phase, flow rate and linearity range were studied to determine the optimum conditions for the assay procedure. Variables were optimized by changing each in turn, while keeping all others constant to obtain the highest resolution.

Mobile phase

A micellar mobile phase has been utilized in this study for the separation of idrocilamide and cinnamic acid. Several modifications in micellar mobile phase composition were studied. These modifications included change of the pH, the concentration of surfactant and type and concentration of co- surfactant.

pH

The pH was changed using increasing volumes of triethyl amine or phosphoric acid. The best pH was found to be 6.0 as it gave the highest number of theoritical plates (Table 1).

The concentration of surfactant

The effect of SDS concentration on retention time and detector response (as peak area) was investigated using mobile phases containing

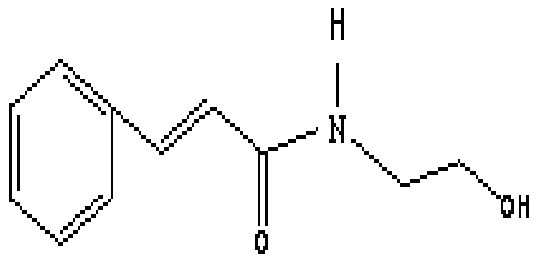


Figure 1: Structural formula of idrocilamide

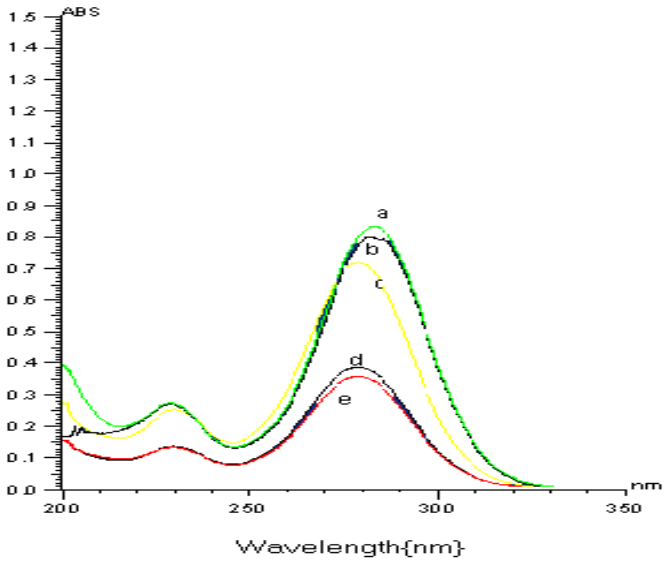


Figure 2: Spectra of idrocilamide(5 µg/mL) before and after degradation
a)Spectrum of idrocilamide in 2 M NaOH on cold.
b)Spectrum of idrocilamide in methanol/water
c)Spectrum of idrocilamide in 1M HCl
d)Spectrum of idrocilamide in 2 M NaOH after degradation (100°C for 45 min).
e)Spectrum of idrocilamide in 1 M HCl after degradation(100°C for 45 min).

SDS concentrations ranging from 0.05 to 0.15 M. It was found that, the increase in the concentration of SDS decreased the retention time of both the drug and its degradation product all over the investigated range. Meanwhile, increasing SDS concentration increased the number of theoretical plates of the drug and its degradation product up to 0.1 M , further increase in SDS concentration up to 0.15 M reduced the number of theoretical plates of the drug and its degradation product (Table 1) Therefore 0.1 M concentration of SDS was used in the study.

The effect of co-surfactant

10% Propanol was replaced with either methanol, ethanol, n- butanol, in an attempt to study the effect of the nature of the co surfactant on the selectivity of the method. The four co-surfactants were found to be equally useful for separation. The number of theoretical plates given as a function of the co-surfactants investigated (Table 1). The concentration of the co- surfactant was investigated using different concentrations within the range of 5-15 %. The retention time decreased with increasing the concentration. A concentration of 10% was found to be suitable as it gave the highest number of theoretical plates (Table 1).

Flow rate

The effect of flow rate on the column efficiency for the separation of the studied drug and its degradation product was studied. The flow rate was changed over the range of 0.5-2mL/min A flow rate of 1mL/min was optimal for a good separation (Table 1).

Specificity

Attempts were made to degrade idrocilamide samples by different ways to assess the stability indicating nature of the method .Solutions of 0.1 mg/mL idrocilamide were prepared in methanol. In both alkaline and acidic media, idrocilamide gave one degradation product with the same retention time at 1.5 min.

Analytical validation

Linearity of the method: After optimizing the conditions, it was found that the peak area and the final concentration was linear over the range 1-10 µg/ml. Linear regression analysis of results gave the following equation:

P.A= 13.57 + 3080C r = 0.9999

Where P.A= Peak area

C= concentration in µg/mL

r= correlation coefficient

The results of the statistical analysis of experimental data for pure samples, such as standard deviation of the slope (s_b) and standard deviation of intercept (s_a) and the standard deviation of residuals (Sy/x) were found to be 0.7, 4.1, 4.8 respectively.

Accuracy and precision

The proposed method was evaluated by studying the accuracy as percent relative error (%Er) and precision as percent relative standard deviation (% RSD) [18]. The results of intraday and interday accuracy and precision for the method are summarized in Table 2.

Limits of detection and quantification

The limit of detection (LOD) was calculated from the calibration curve according to the formula LOD= 3.3 S_a/b [18], with Sa being the standard deviation of the intercept of the regression line, b being the slope of the calibration curve. The limit of quantification, defined here as LOQ= 10 S_a/b were determined on the basis of standard deviation of response and slope.

Application to cream

The proposed method was successfully applied for the determination of idrocilamide in cream. The average percentage recovery was 100.74 ± 0.93. The results shown in Table 3 are in good agreement with those obtained by the comparison method [3].The latter involves the use of column (20 cm × 5 mm) of octadecylsilane (ODS) with a mobile phase consists of aqueous 60% methanol with flow rate of 1mL/min. and detection at 280 nm. The proposed method is fairly sensitive since it can measure down to 1.0 µg/ mL. The comparison method is linear over the range 4-16 µg/ml.

Application to biological fluids

The high sensitivity attained by the proposed method allows determination of idrocilamide in biological fluids without extraction Figure 3. The reported maximum plasma concentration was 2.58 µg/ml [19]. Table 4 shows the results of recovery studies from the corresponding calibration curve for spiked plasma and urine.

Experimental Parameters		N_1	N_2	K'_1	K'_2	α
pH	3	1115	991	2.1	0.45	4.7
	3.5	1775	1100	2.5	0.41	6.1
	4	1994	1172	2.21	0.5	4.42
	4.5	1226	1180	2.6	0.51	5.2
	5	2259	1215	2.44	0.51	5.1
	5.5	2366	1246	2.55	0.52	4.7
	6	2444	1250	2.44	0.52	4.7
	6.5	1354	1190	2.15	0.51	4.2
	7	1268	1115	1.5	0.51	3.1
SDS conc. (Mole/L)	0.05	1443	1240	3.5	0.9	3.11
	0.08	2366	1246	2.55	0.52	4.7
	0.1	2444	1250	2.2	0.42	5.2
	0.12	1665	1120	1.8	0.38	4.7
	0.15	1323	1028	1.5	0.3	5
Type of co –Surfactant.	Methanol	1804	1185	2.33	0.48	4.9
	Ethanol	1999	1230	2.35	0.5	4.7
	n-Propanol	2366	1246	2.55	0.52	4.7
	n-Butanol	1745	1200	2.41	0.52	4.6
Conc. of propanol	6%	2106	1233	2.8	0.8	3.2
	8%	2366	1246	2.55	0.52	4.7
	10%	2444	2250	2.3	0.4	5.75
	12%	2038	1170	1.86	0.35	5.3
	15%	1462	1030	1.6	0.3	5.33
Flow rate (ml/min)	0.5	2138	980	5.74	2	2.8
	0.8	2285	1190	4.1	1.5	2.73
	1	2444	1250	2.55	0.52	4.7
	1.2	1816	1200	2	0.35	5.71
	1.5	1998	1140	1.56	0.23	6.8

N1, N2 :The number of theoretical plates of cinnamic acid and idrocilamide

K'_1, K'_2: The capacity factors of cinnamic acid and idrocilamide

α: The selectivity factor

Table 1: Effect of different experimental parameters on the column efficiency for the separation of idrocilamide and its degradation product

Conc.added µg/mL	Conc.found (µg/mL)	% Found	% RSD	% Er
Intraday				
1	0.998	99.81±0.10	0.1	0.058
5	4.9895	99.79±0.12	0.12	0.069
10	9.996	99.96±0.10	0.1	0.058
Interday				
1	1.01	100.10±0.26	0.26	0.15
5	4.995	99.90 ±0.30	0.3	0.17
10	10.02	100.2±0.2	0.2	0.11

Each result is the average of three separation determinations

Table 2: Intraday and inter day precision for the proposed method

Preparation	Proposed method			Comparison method [3]	
	Amount taken, µg/mL	Amount found, µg/mL	% Found*	Amount taken, µg/mL	% Found*
1)Srelane cream 5%	1	0.995	99.5	4	99.2
	3	3.012	100.4	8	99.1
	7	6.937	99.1	12	99.8
	10	10.03	100.3	16	100.1
Mean±SD			99.83±0.40		99.60±0.48
t-test		0.74	(2.45)**		
F-test		1.44	(9.277)**		

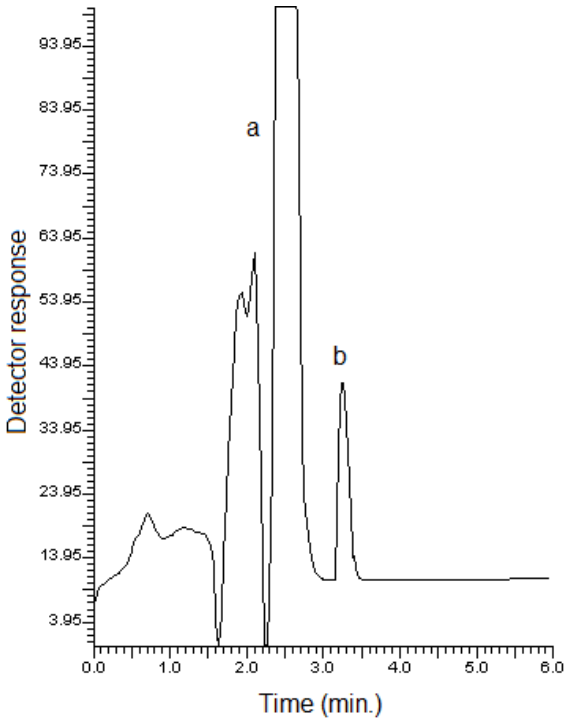
Minapharm pharmaceuticals, Cairo,Egypt,batch No.6CE0246

Table 3: Application of the proposed and comparison methods to determination of idrocilamide in commercial cream

Sample	Proposed method			Comparison method [3]	
	Amount added, µg/mL	Amount found, µg/mL	% Recovery	Amount added, µg/mL	% Recovery
1) Plasma	1	1	100	4	100
	5	5.01	100.2	8	100.5
	10	9.96	99.6	12	99.14
Mean±SD			99.93±0.31		99.88±0.69
t-test		0.26	(2.132)**		
F-test		4.95	(19.00)**		
2) Urine	1	1	100	4	100
	5	5.02	100.4	8	99.9
	10	9.99	99.9	12	100.7
Mean±SD			100.10±0.26		100.20±0.44
t-test		1.15	(2.132)**		
F-test		2.86	(19.00)**		

** The value of tabulated t and F, (at p = 0.05) [13]

Table 4: Determination of idrocilamide in biological fluids by the proposed and comparison methods

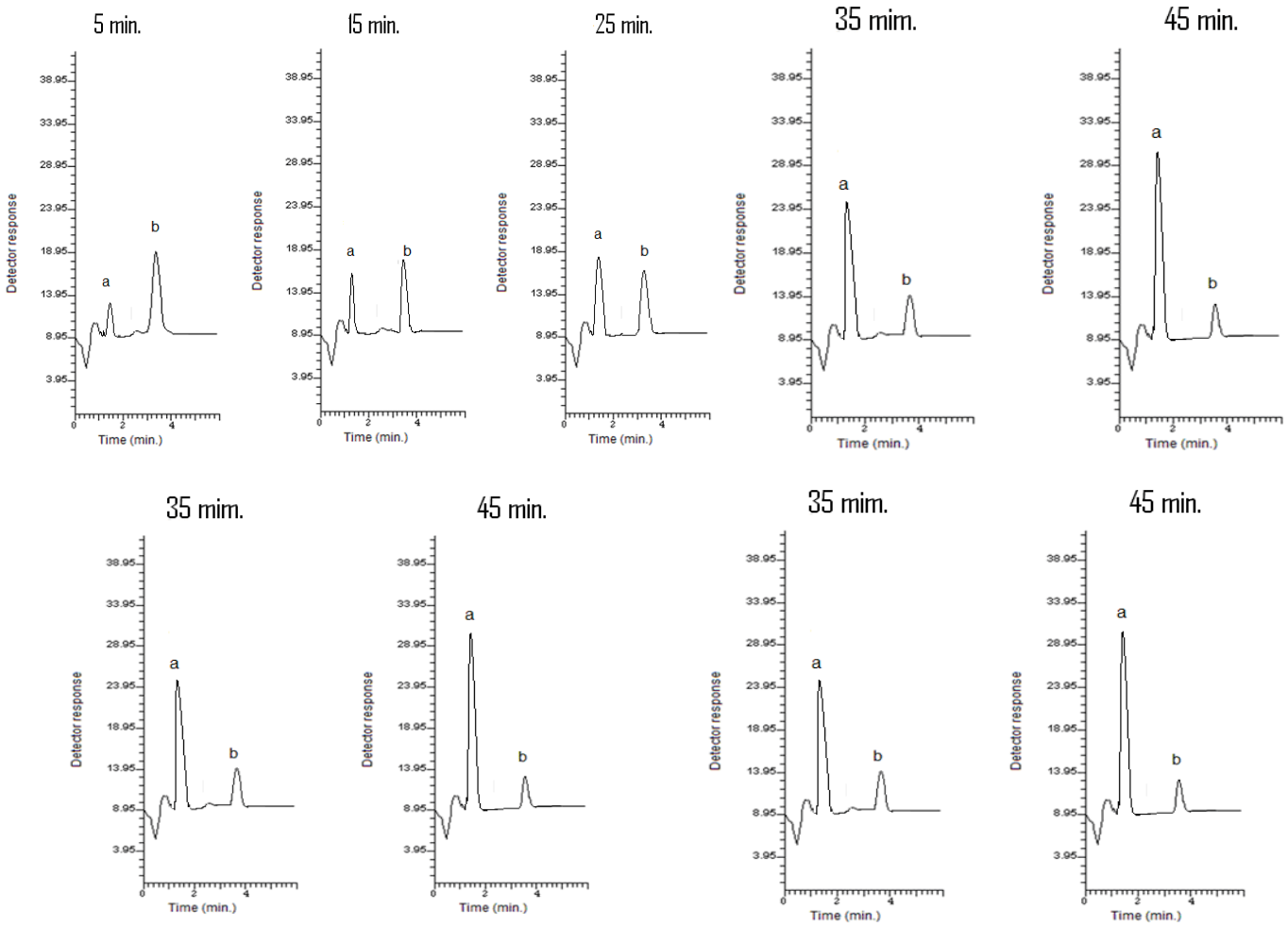


a: Plasma peak
b: idrocilamide

Figure 3: Chromatogram of idrocilamide (5µg/mL) in spiked plasma

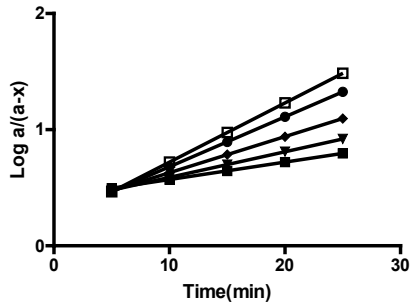
Medium	Temperature(˚C)	K(min^{-1})	$t_{1/2}$(min.)	Ea(K.Joule)
Acidic degradation	60	0.0206	33.7	15.3
	70	0.0223	30.7	13.3
	80	0.0251	27.6	8.5
	90	0.0283	24.5	7.4
	100	0.032	21.66	
Alkaline degradation	60	0.0174	39.83	16.1
	70	0.0185	37.4	14.5
	80	0.021	33.1	10.35
	90	0.024	28.9	8.5
	100	0.0276	25.1	

Table 5: Degradation rate constant (K) and half life time ($t_{1/2}$) for idrocilamide



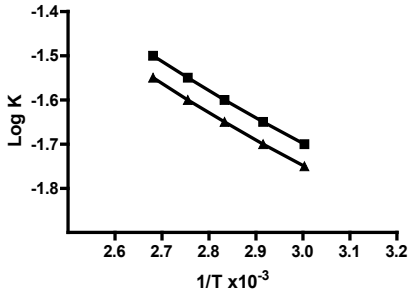
a)degradation product b)Idrocilamide (5µg/mL)
using mobile phase consisting of 0.1 M SDS,10 % propanol and 0.3 % triethyl amine and 0.02 M phosphoric acid (pH 6) with UV detection at 277 nm.

Figure 4: Chromatogram showing the degradation of idrocilamide at 100º C for different time intervals.



- ■ Y = 0.42 + 8.94 x10^{-3} t (60 °C) r = 0.997
- ▼ Y = 0.36 + 9.7 x10^{-3} t (70 °C) r = 0.997
- ◆ Y = 0.32 + 0.011 t (80 °C) r = 0.998
- ● Y = 0.24+ 0.0123 t (90 °C) r = 0.998
- □ Y = 0.22 + 0.014 t (100 °C) r = 0.998

Figure 5: Semilogarithmic plot of the amount remaining of idrocilamide 5µg/mL versus different heating times



Arrhenius plot of acidic degradation
Y=-0.45-701 1/T

Arrhenius plot of alkaline degradation
Y=-0.33-770.3 1/T

Figure 6: Arrhenius plot for the degradation of idrocilamide

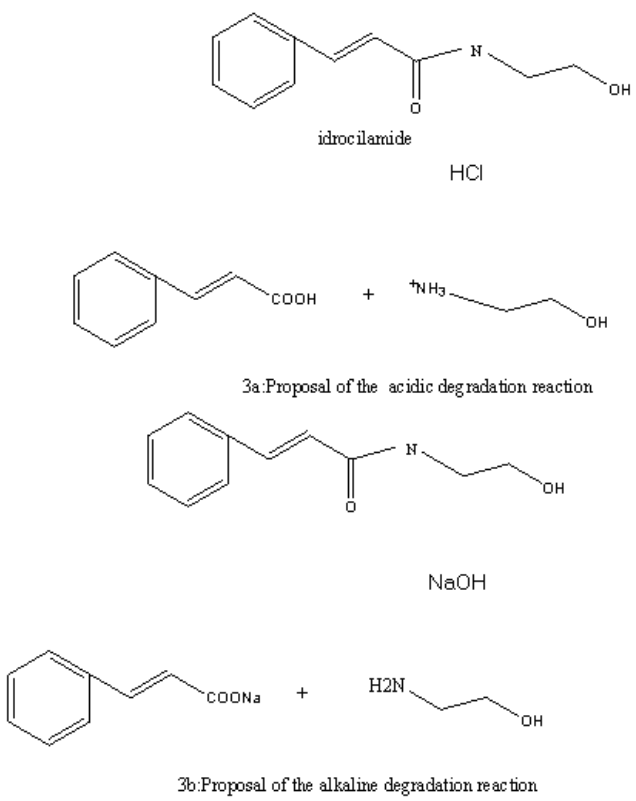


Figure 7: Proposal scheme of the degradation reaction of idrocilamide in both acid and alkaline media

Degradation kinetics

Figure 4 shows the chromatogram of idrocilamide after being subjected to degradation.The degradation was found to be temperature dependent (Figure 4). At the selected temperatures settings (60-100°C),the degradation followed pseudo-first order kinetics (Figure 5). The apparent first order degradation rate constant and the half life time at each temperature were calculated (Table 4). Plotting log K values *vs* 1/T,the Arrhenius plot [20] was obtained (Figure 6). The activation energy was calculated and found to be 14.75 K.J/mole in case of alkaline degradation and 13.42 K.J /mole in case of acidic degradation .These values are in accordance with the reported values for amide group [20]. It is postulated that the degradation process involve the amide linkage in both acidic and alkaline media (Figure 7).

Stability

The stability of the methanolic sample solutions at room temperature (25°C) for 24 hour after preparation,was verified by reassaying them. There is no indication of any decomposition of idrocilamide in the samples for one week.

Chromatographic performance

Two defined symmetrical peaks were obtained upon measuring the UV response of the eluate under the optimum experimental parameters (Figure 6). Idrocilamide peak was obtained at retention time of 3.2 min and the degradation product at 1.5 min.

Conclusion

The present investigation confirms that the use of micellar mobile phases in HPLC provides selectivity and separation efficiency comparable to conventional reversed phase HPLC systems for the determination of idrocilamide. The proposed method provides a stability indicating method for the determination of idrocilamide in presence of its degradation products offering additional advantages over comparison methods. The proposed method can be used in routine analysis of idrocilamide pharmaceutical preparations and biological fluids.

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