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Different Spectrophotometric and TLC-Densitometric Methods for Determination of Diclofenac Na and Lidocaine HCl

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ABSTRACT
Diclofenac sodium (DIC) Diclofenac Na is a non-steroidal anti-inflammatory drug (NSAID), which is used for relieving pain and inflammation in various conditions, while Lidocaine HCl (LID) is a local anathesetic which acts by preventing the generation and transmission of impulses along nerve fibers and nerve ending. In this work, three accurate, precise and specific spectrophotometric and chromatographic methods have been developed and validated for determination of these drugs in their bulk powder and pharmaceutical dosage form. The developed methods are Ratio subtraction spectrophotometric (Method I), mean centering of ratio spectra MCR (Method II) and TLC-Denitometric (Method III), In method (I) where LID can be determined by dividing the spectrum of the mixture by the spectrum of DIC (as a devisor) followed by subtracting the constant absorbance value of the plateau region, then finaly multiplying the produced spectrum by the spectrum of the divisor, while DIC concentration can be determined directly at 282 nm. In method (II) absorption spectra of each drug were recorded, divided by suitable divisor and the obtained ratio spectra were then mean centered. Method (III) is TLC – Densitometric method that depends on quantitative separation of DIC and LID on TLC plates using ethyl acetate: chloroform: methanol: ammonia (5:3.3:1.5:0.2 by volume) as mobile phase and scanning at 203 nm.
The developed methods were validated according to ICH guidelines demonstrating good accuracy and precision. The results were statistically compared with those obtained by reported method and no significant difference were found between them.

Keywords: Diclofenac Na, Lidocaine HCl, Ratio subtraction, Mean centering of ratio spectra spectrophotometry, TLC-densitometry.

1. Introduction
Diclofenac sodium (DIC) is a phenyl acetic acid derivative [1] (Figure 1a) and nonsteroidal anti-inflammatory agent (NSAID), it is administered in different conditions such musculoskeletal and joint disorders. Also it used in eye drops for prevention of intra- operative miosis during cataract extraction [2]. Lidocaine HCl (LID) Lidocaine Hcl is 2-(Diethylamino)-N-(2, 6-dimethylphenyl)-acetamide hydrochloride [1] Fig.1 (b). Lidocaine is a local anesthetic that works by causing temporary numbness/loss of feeling in the skin and mucous membranes [2].

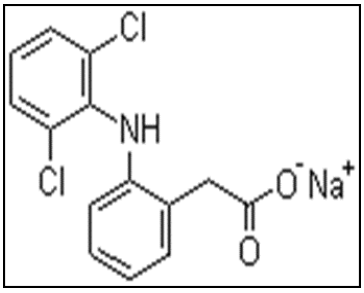

Fig a

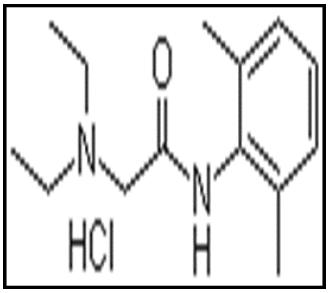

Fig b

Fig 1: Chemical structures of (a) Diclofenac Na, (b) Lidocaine HCl

The two studied drugs have been formulated in combined dosage form called Olfen®ampoules. The combined formulation is recommended to be used to ensure that olfen injections less painful and have a better local tolerance. On reviewing the literature in hand, it was found that both. British [3] and United States [4] Pharmacopoeias analyzed DIC in its raw material by potentiometric non-aqueous titration and determined it in its dosage forms by RP-HPLC method [3, 4]. DIC has been also analyzed along with other drugs by different methods such as Spectrophotometric [6-9], multivariate calibration [10], spectroflurimetric [11, 12], TLC-Densitometric [13-15], HPLC [16-19], capillary electrophoretic [20, 21] and electrochemical [22] methods.

On reviewing the literature in hand, *The British Pharmacopoeia* (BP; 4) and the *United States Pharmacopeia* (USP; 5) suggest several procedures for the assay of LID powder and dosage forms. Most BP procedures depend on titrimetry for LID in powder form. HPLC was described for the assay of the ointment form. On the other hand, HPLC is predominant in the USP monographs of LID in powder formand its dosage forms, while titrimetric procedures are used for the semisolid topical preparations (ointment and jelly) [3, 4]. LID has been also analyzed by different methods such as Spectrophotometric [23], multivariate calibration [2], GC [25], TLC-Densitometric [26, 27], HPLC [28-31] and capillary electrophoretic [32] methods.

The binary mixture of DIC and LID has been analyzed by two reversed phase HPLC methods for the simultaneous determination of DIC and LID in binary mixture and pharmaceutical formulation [33, 34].

This work concerns with development and validation of three spectrophotometric methods and TLC-Densitometric one for determination of the suggested drugs in their raw materials, synthetic mixtures and combined dosage form. The suggested methods have the advantages of saving time and cost when compared to the published HPLC methods [33, 34]. They don't need high cost instruments or chemicals. Also the proposed TLC-Densitometric method has the advantage of being more sensitive than other developed method and then the published RP-HPLC methods.

2. Experimental

2.1. Instruments

- Adouble beam UV-visible spectrophotometer (SHIMADZU, Japan) model UV-1601 PC with quartz cell of 1 cm and UV-PC personal software version 3.7 was used. The spectral band width is 2 nm and wavelength-scanning speed 2800 nm/min. All data analysis was performed using PLS-Toolbox 2.0 [35] running under MATLAB®, version 6.5 [36].
- A UV lamp with short wavelength 254 nm UV Lamp (Viber Lourmat, Marine LA VALLEE Cedex 1, France).\
- A TLC scanner 3 densitometer (camage, Muttenz, Switzerland), the following requirements are taken into consideration.
- Slit dimension: 5x0.2 mm, scanning speed: 20mm/s, spraying rate: 10 sμL^{-1}, data resolution: 100 μm/step.
- TLC plates (20x20 cm) coated with silica gel 60F_{254} (Fluka, Sigma-Aldrich Chemie GmbH, Germany). A sample applicator for TLC Linomt IV with 100 μL syring (Cama, Muttenz, Switzerland)

2.2. Materials

2.2.1. Pure standards

DIC/LID were kindly applied by medical union co. for pharmaceutical, Abn sultan, Ismailia, Egypt. Their purity was found to be 100.11% and 100.05% respectively according to BP (1 2) methods

2.2.2. Pharmaceutical dosage forms

Olfen® ampules (Batche No.110959) each ampule labeled to contain 75 mg and 20 mg of diclofenac Na and Lidocaine Hcl respectively, manufactured by mepha Pharmaceutical preparation containing the studied drugs were purchased from the local market.

2.2.3. Solvents

Methanol HPLC grade (CHROMASOLVE®, Sigma -Aldrich Chemie GmbH, Germany).

2.2.4. Standard solutions

a) Standard stock solution of DIC and LID were prepared in methanol in the concentration of 1 mg mL^{-1}.
b) Standard working solutions of DIC and LID were prepared in methanol in the concentration of 0.1 mg mL^{-1}.

2.3. Procedures

2.3.1. Spectral characteristics and wavelengths selection

The absorption spectra of 8 μg mL^{-1} of each of DIC and LID were recorded over the range of 200-400 nm using methanol as a blank. The spectra were observed for selecting of the suitable wavelengths for Ratio subtraction and mean centering of ratio spectra spectrophotometric methods. Figure.2

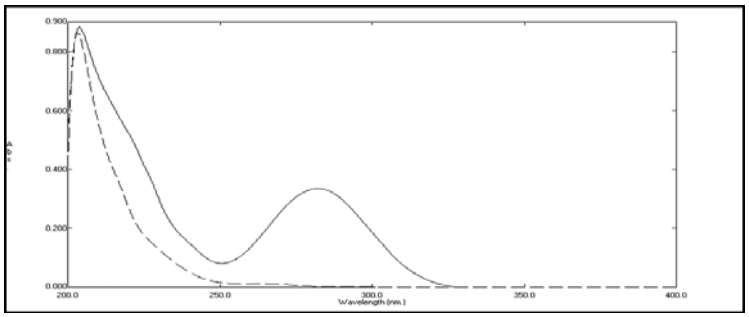

Fig 2: Zero order absorption spectra of 8 μg mL^{-1} of each of Diclofenac Na (__) and Lidocaine Hcl (_ _), using methanol as a blank.

2.3.2. Construction of calibration curves

2.3.2.1. Ratio subtraction method

aliquots containing 80-320 µg and 20-100 µg of DIC and LID, respectively, were accurately transferred from their respective working standard solutions (0.1 mg mL^{-1}) into two separate sets of 10mL volumetric flasks; and the volume was completed using methanol to obtain final concentrations of 8-32 µg mL^{-1} and 2-10 µg mL^{-1} ,for DIC and LID, respectively. The prepared solutions were scanned in the range of 200 – 400 nm and the absorbance values at 203 nm for LID and at 282 nm for DIC respectively were measured. The calibration curves relating the absorbance of each curve at the selected wavelength to the corresponding drug concentrations were constructed and the regression equation corresponding to each calibration curve was calculated. The spectra of prepared mixtures were divided by the spectrum of 16 µg. ml^{-1} of DIC (as a divisor). The absorbencies in the plateau at λ. above 282 nm were subtracted from the corresponding spectra of the mixture and then the produced spectra were multiplied by the spectrum of the divisor. LID concentration was determined at its λ_{max} 203 nm using its corresponding regression equation while DIC was measured at its λ_{max} 282 nm in the zero-order spectrum of the mixture and its concentration determined from its corresponding regression equation.

2.3.2.2. Mean centering of ratio spectra (MCR) method

Aliquots containing 80-320 µg and 20-100 µg of DIC and LID, respectively, were accurately transferred from their respective working standard solutions (0.1 mg mL^{-1}) into two separate sets of 10 mL volumetric flasks; and the volume was completed using methanol to obtain final concentrations of 8-32 µg mL^{-1} and 2-10 µg mL^{-1}, for DIC and LID, respectively. For measuring DIC, The scanned spectra of DIC in the range of 205-325 nm were divided by standard spectrum of 4 µg mL^{-1} LID and the obtained ratio spectra were mean centered.

For measuring LID concentration, the previously scanned spectra in the range of 200-245 nm were divided by the standard spectrum of 8 µg mL^{-1} DIC and the obtained ratio spectra were then mean centered.

The values of the mean centered ratio spectra amplitudes at 245 and 223.2 nm for DIC and LID, respectively, were plotted against their corresponding concentrations and the regression equations were computed.

2.3.2.3. TLC-Densitometric method

Accurate volumes of DIC and LID in the range of 4-40 and 1-10 µg/band were separately transferred from their respective stock standard solutions (1 mg mL^{-1}), applied in triplicates to the prewashed TLC plates. 10 µL of each prepared sample were applied in triplicates as bands of 6 mm width on TLC plates (20x10 cm with 250 µm thickness) using a Camag Linomat IV applicator. The bands were applied at 5 mm intervals and 10mm from the bottom edge of the plate. Linear ascending chromatogram development to a distance of 8 cm was performed in a chromatographic tank previously saturated for 30 minutes with a developing system consisted of ethyl acetate: chloroform: methanol: ammonia (5:3.3:1.5:0.2 by volume)at room temperature. The applied bands were scanned at 203 nm and the calibration curves were constructed by plotting the integrated peak area versus the corresponding concentrations of each drug and the regression equations were computed.

2.3.3. Analysis of laboratory prepared mixtures

Different laboratory prepared mixtures containing different ratios of (DIC and LID) were prepared and the procedures under construction of calibration curves for each method was followed. Concentrations of DIC and LID in the prepared samples were calculated from the computed regression equations.

2.3.4. Analysis of pharmaceutical dosage forms

The contents of 10 Olfen ampoules were mixed well and accurately measured volume equivalent to 25 mg LID and 100 mg of DIC was transferred into 100-ml volumetric flask, diluted to the mark with methanol (HPLC grade) and further dilution were ions of 25 µgml^{-1} and 100 µg.ml^{-1} of LID and DIC, respectively. The procedure of each method was followed and the concentration of DIC and LID was calculated from the corresponding regression equation.

2.3.4.1. Recovery studies

Recovery studies were carried were carried out by applying the standard addition technique.

3. Result and discussion

The combination under investigation is used in inflammatory and painful conditions such as rheumatoid arthritis, osteoarthritis, postoperative conditions. The combination of DIC and LID in Olfen® ampoule to reduce the feeling of pain at the injection site. On the other hand, the literature showed only two reversed phase HPLC methods for the simultaneous determination of some pharmaceuticals containing DIC and LID in binary mixture and pharmaceutical formulation Due to the wide application of the studied combination and due to the draw backs of the previous published methods for resolving the studied mixture, we aimed in this work to develop and validate accurate, precise, sensitive and selective spectrophotometric and chromatographic methods for measuring the studied drugs in their combined formulation without preparing or treating the sample

3.1. Ratio subtraction spectrophotometric method

First, the linearity of LID was determined in the concentration range 2-10 µg ml^{-1} at 203 nm in the zero order spectra. Different divisor concentrations (10, 12 and 16 µg ml^{-1}) were tried. The divisor concentration 16 ug ml^{-1} of DIC was found to be the best regarding accuracy and precision when the method was used for calculation of LID concentration in its laboratory prepared mixtures.

Second, the spectrum of the mixture of LID and DIC in methanol was divided by the spectrum of the divisor (16 ug mL^{-1} of DIC}. The value of the absorbance in the plateau region at λ above282 nm was subtracted from the spectrum of the divided mixture; the obtained spectrum was then multiplied by the spectrum of the divisor as shown in Figure3. Finally, LID concentration was measured from the last spectrum obtained at 203 nm, while DIC concentration was determined from zero-order spectrum at its λ_{max} 282 nm.

Linear correlation were obtained between absorbance at 282 nm for DIC and its concentration in the range 4-40 ug mL^{-1} and at 203 for LID and its concentration in the range of 2-10 ug.mL^{-1} from which the regression equation were calculated and found to be :

$A_{DIC}=0.0425C_{DIC}+0.0014r_{DIC} = 0.9995$For DIC at 282nm

$A_{LID} = 0.0894\ C_{LID} + 0.136 r_{LID} = 0.9998$For LID

at 203 nm where A_{DIC} and A_{LID} are the absorbance values of DIC and LID respectively, C_{DIC} and C_{LID} are the concentrations of DIC and LID in µg ml^{-1} respectively and r_{DIC} and r_{LID} are the correlation coefficients of DIC and LID respectively.

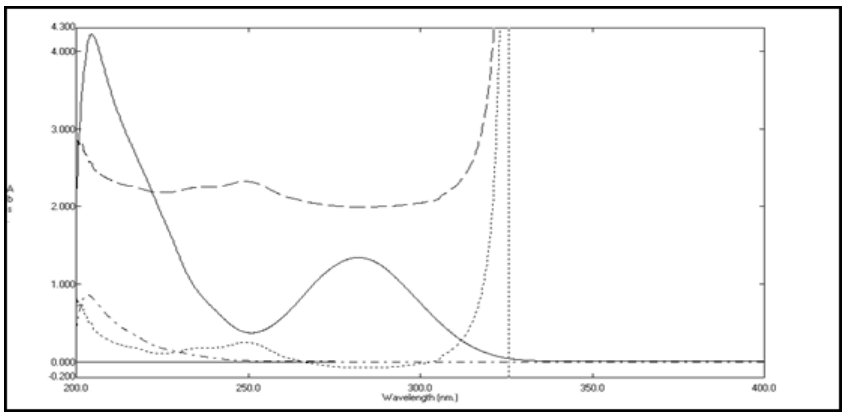

Fig 3: Absorption spectra of DIC and LID mixture (_ _) mixture after division by the devisor (_ _ _) mixture after subtraction of the constant (...) and mixture after multiplication by the devisor (-.-.-) using methanol as a blank.

3.2. Mean centering of ratio spectra (MCR) spectrophotometric method

The developed MCR method is based on the mean centering of ratio spectra. The method was developed and illustrated by Afkhami and Bahram [37], on applying this method, we do not need for spectral derivatization steps and hence method sensitivity is enhanced. In order to optimize the developed MCR method, different parameters were tested. The wavelength range taken was found to have agreat effect on the obtained mean centering ratio spectra, different wavelength ranges were tested. The best results were obtained when using the wavelength range from (205-325 nm) for DIC, while for LID from (200-245 nm). Also the effect of divisor concentration on the selectivity of the method was checked by testing several concentrations each of DIC and LID. The best results regarding sensitivity and selectivity were obtained by using 8 and 4 µg mL^{-1} each of DIC and LID respectively as divisors.

Beer's Lambert law was obeyed in the range of 8-32 µg mL^{-1} at 245 nm for DIC, while for LID in the range of 2-10 µg mL^{-1} at 223.2 nm Figures (4, 5). The regression equations for the proposed method were calculated and found to be:

$P_{DIC}=0.1085C_{DIC}–0.0701$ r= 0.9997for DIC at 245 nm

$P_{LID}=0.4551C_{LID}$ –0. 207 r= 0.9997 for LID at 223.2 nm

Where, P is the peak amplitude at the selected wavelengths, C is the concentration in µg $mL^{-1}$and r is the correlation coefficient.

3.3. TLC-densitometric method

The TLC-Densitometric technique was successfully applied for the determination of DIC and LID in pure form and in pharmaceutical formulations. This method offers a simple way to quantify directly on TLC plate by measuring the optical density of the separated bands.

In order to obtain optimum separation among the studied Drugs, different trials have been carried out to reach the optimum developing system, scanning wavelength, band dimension and slit dimension. Firstly Developing system such as ethyl acetate: chloroform (6:4, v/v), ethyl acetate: chloroform: methanol (5:4.5:0.5, by volume), ethyl acetate: chloroform: methanol (5:3.5:1.5, by volume), ethyl acetate: chloroform: methanol: ammonia (5:3.3:1.5:0.2 by volume) were tested. On using the first system bad results among DIC and LID has been observed, while using the following two systems, DIC and LID had good separation but DIC peak was tailed. Upon addition of ammonia, a noticed improvement of DIC tailing. The best results concerning both peak separation and symmetry were obtained upon using the last system, this system was found to give compact, sharp and symmetrical spots for DIC and LID with suitable R_f values Also.

Which gave good R_f values for both drugs where R_f values were 0.15 and 0.85 for DIC and LID, respectively, Figure (6). Different scanning wavelengths were tried such as 203, 254, and 280 nm; the best scanning wavelength was 203 nm which showed good sensitivity with minimum noise for all the studied drugs.

Different band dimensions were tested to obtain sharp and symmetrical peaks. The optimum band width was 6 mm with 8.9 mm inter-space between bands.

The slit dimensions of scanning should ensure complete coverage of band on the scanned track without interference of adjacent bands. Different slit dimensions were tried, where 6 mm × 0.45 mm proved to be the slit dimensions of choice. The calibration curves were constructed by plotting the integrated peak area versus the corresponding concentrations in the concentration range 4-40 µg/ band for DIC and 1-10 µg/ band for LID. The regression equations were computed and found to be:

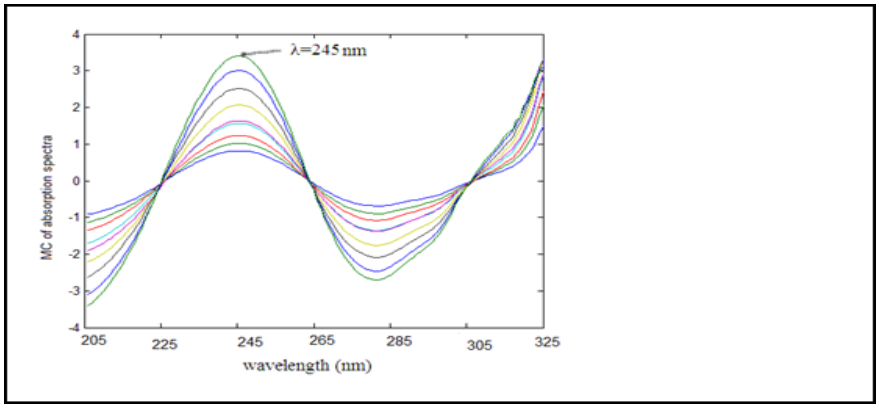

Fig 4: Mean centered ratio spectra of DIC (8-32 µg mL^{-1}) using 4 µg mL^{-1} of LID as a divisor and methanol as solvent.

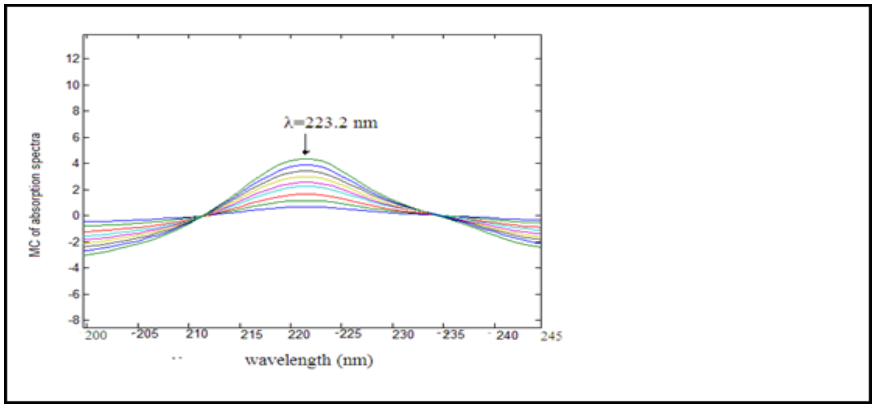

Fig 5: Mean centered ratio spectra of LID (2-10 µg mL^{-1}) using 8 µg $mL^{-1}$of DIC as a divisor and methanol as solvent.

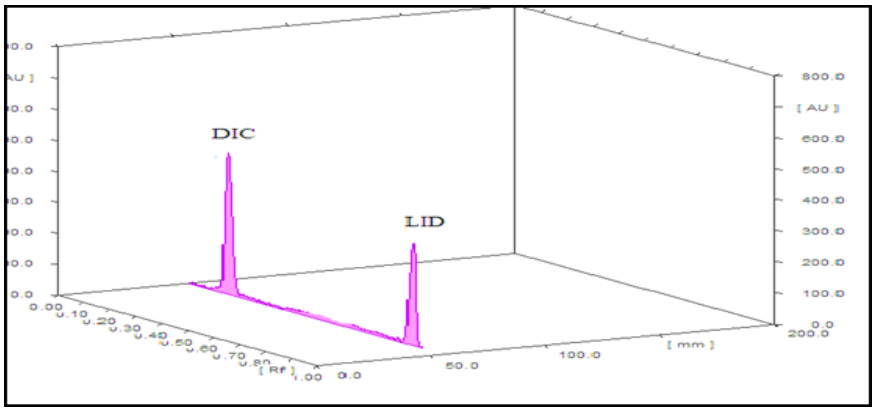

Fig 6: TLC Densitogram of mixture of, Diclofenac Na, Lidocaine HCl using developing system of: ethyl acetate- chloroform-methanol-ammonia (5:3.3:1.5:0.2, by volume)

$Y_{DIC}=633.62C_{DIC}+17008$ r = 0.9997
$Y_{LID}=953.73C_{LID}+5605.7$ r = 0.9997

Where, Y is the peak area, C is the concentration in µg/ band and r is the correlation coefficient. The developed spectrophotometric and chromatographic methods were also applied for determination of DIC and LID in Olfen® ampoules without interferences from suppositories excipients and satisfactory results were obtained. Standard addition technique was performed in order to asses the validity and accuracy of the methods where good percentage recoveries were obtained indicating no interference from excepients Table (2). The results obtained by applying the proposed methods were statistically compared with those obtained by applying the reported HPLC method [33] for determination of the proposed drugs in their pure forms and no significance differences were obtained between them Table (3). The test ascertains that the proposed methods are as precise and accurate as the reported HPLC method [34] and are comparable to one another.

Table 1: Regression and analytical parameters of the proposed Ratio subtraction, MCR spectrophotometric and TLC-Densitomtric methods for determination of Diclofenac Na and Lidocaine HCl.

Parameters	Ratio subtraction spectrophotometric method		Mean centering of ratio spectra (MCR) spectrophotometric method		TLC-Densitometric Method	
	DIC	LID	DIC	LID	DIC	LID
Range	8-32 µgml^{-1}	2-10 µgml^{-1}	8-32 µgml^{-1}	2-10 µgml^{-1}	4-40 µg/ band	1- 10 µg/ band
Slope	0.043392	0.0894	0.01085	0.4551	633.62	953.73
Intercept	0.014	0.136	-0.0701	-0.207	17008	5605.7
Correlation coefficient	0.9995	0.9998	0.9997	0.9997	0.9997	0.9997
Accuracy (%)	100.17	100.06	99.48	100.38	99.80	99.94
Specificity± RSD	99.57 ± 0.541	100.79± 0.965	99.71 ±0.668	100.58 ±0.859	———	———
Precision Repeatability RSD	0.652	0.511	1.153	1.682	0.612	0.824
Intermediate precision RSD	0.714	0.651	1.235	1.734	0.745	0.931

Table 2: Quantitative determination of DIC and LID in Olfen® ampoules by the Ratio subtraction, MCR spectrophotometric and TLC-Densitomtric methods and application of standard addition technique.

Olfen® ampoules Batch No. 110959						
	Ratio subtraction spectrophotometric method		MCR		TLC-densitomtriy	
	DIC	LID	DIC	LID	DIC	LID
Taken µg mL^{-1}	15	4	15	4	15	4
Mean[a] %	100.40	98.50	100.53	104.80	99.70	99.80
Added µg mL^{-1}	8.00	3.00	8.00	3.00	5.00	2.00
	10.00	4.00	10.00	4.00	10.00	3.00
	15.00	5.00	15.00	5.00	15.00	4.00
% recovery[b]	98.86	98.67	99.13	99.33	102.00	96.60
	98.70	98.75	99.10	99.25	101.90	97.67
	98.53	101.60	98.73	100.60	99.80	100.25
Mean ±SD	98.69 ±0.165	99.67 ±1.669	98.98 ±0.223	99.72 ±0.757	101.27 ±1.23	98.17 ±1.876

a – average of 6 determinations b- Average of 3 determinations

Table 3: Statistical analysis of the proposed Ratio subtraction, MCR, spectrophotometric and TLC- Spectrodensitometric methods and reported one for determination of DIC and LID in their pure forms

Parameters	Ratio subtraction spectrophotometric method		MCR		TLC-densitomtriy		Reported HPLC Method(33)	
	DIC	LID	DIC	LID	DIC	LID	DIC	LID
Mean %	100.17	100.06	99.48	100.38	99.80	99.94	100.12	100.69
SD	1.008	0.960	1.062	1.003	0.833	1.196	1.098	0.965
N	7	7	7	7	7	7	7	7
Student t-test (2.22)*	0.38	0.83	0.67	0.85	0.89	0.059	___	____
F- value (5.05) *	1.42	3.89	1.42	3.74	1.36	1.17	_____	_____

3.4. Method validation

Validation of the methods was carried out according to ICH recommendation [38].

3.4.1. Linearity and range

The calibration range for DIC and LID was established through considerations of the practical range necessary The concentrations were calculated from the corresponding regression equations and the results are shown in Table (1). according to adherence to Beer-lambert's law to give accurate, precise and linear results. Linearity ranges of DIC and LID are shown in (Table 1).

3.4.2. Accuracy

Accuracy of the proposed methods was calculated as the percentage recoveries of pure samples of the studied drugs.

Accuracy was further assessed by applying the standard addition technique to Olfen® ampoules, where good recoveries were obtained revealing no interference from excipients Table(2).

3.4.3. Precision

3.4.3.1. Repeatability

Three concentrations (8, 12 and 16 µg mL^{-1} of DIC and 2, 4 and 6 µg mL^{-1} of LID) were analyzed three times intra-daily using the proposed methods. Good results and acceptable relative standard deviations (RSDs) were obtained, Table (1).

3.4.3.2. Intermediate precision

The previous procedures were repeated inter-daily on three different days for the analysis of the chosen concentrations. Good results and acceptable RSDs values were obtained, Table (1).

3.44. Selectivity

Selectivity of the proposed methods was assessed by the analysis of different synthetic laboratory prepared mixtures containing different ratios of (DIC and LID) within their linearity ranges. Satisfactory results are shown in Table (1).

3.4.5. Robustaness

The recommended TLC- Densitometric method was found to remain unchanged with small changes in method parameters e.g.: changing ammonia ratio in the developing system ± 0.02 mL, changing saturation time ± 5 min and changing the scanning wavelength ± 1 nm. Which assessed the robustness of the validated method.

3.4.6. System suitability testing parameters

When system suitability testing was done, acceptable results were obtained and the peaks information was given in The resolution (R s) and selectivity factors (α) values were above 1 and 1.5, respectively, which ensured good separation of each component from the other Table(4)

Table 4: Parameters of system suitability of the developed TLC-Densitometric method.

Parameters	DIC	LID
Symmetry factor	1	1.04
Resolution(Rs)	6.6	
Capacity factor(k')	0.85	
Selectivity (α)	4.08	

4. Conclusion

The developed methods have advantages over the published methods in being more simple, rapid, cost effective and data processing steps are not time consuming. Spectrophotometric methods can be regarded as a useful alternative to chromatographic techniques in the routine quality control analysis of pharmaceutical formulations allowing rapid determination at relatively low cost. The advantages of TLC-densitometric method is its ability to determine the studied drugs using one and the same developing system and scanning wavelength, several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost. The developed methods can be easily adopted for routine quality control analysis of DIC and LID.

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