Research Article

Drug Testing and Analysis

Received: 11 August 2010 Revised: 23 September 2010 Accepted: 24 September 2010 Published online in Wiley Online Library: 29 December 2010

(www.drugtestinganalysis.com) DOI 10.1002/dta.213

Stability-indicating spectrophotometric and spectrodensitometric methods for the determination of diacerein in the presence of its degradation product

Marianne Nebsen,[a] Mohamed K. Abd El-Rahman,[a]* Maissa Y. Salem,[a] Amira M. El-Kosasy[b] and Mohamed G. El-Bardicy[a]

Three sensitive, selective, and precise stability-indicating methods for the determination of the novel osteoarthritis drug, diacerein (DIA) in the presence of its alkaline degradation product (active metabolite, rhein) and in pharmaceutical formulation were developed and validated. The first method is a first derivative (D_1) spectrophotometric one, which allows the determination of DIA in the presence of its degradate at 322 nm (corresponding to zero crossing of the degradate) over a concentration range of 4–40 μg/mL with mean percentage recovery 100.21 ± 0.833. The second method is the first derivative of the ratio spectra (DD_1) by measuring the peak amplitude at 352 nm over the same concentration range as (D_1) spectrophotometric method, with mean percentage recovery 100.09 ± 0.912. The third method is a TLC-densitometric one, where DIA was separated from its degradate on silica gel plates using ethyl acetate : methanol : chloroform (8 : 1.5 : 0.5 v : v : v) as a developing system. This method depends on quantitative densitometric evaluation of thin layer chromatogram of DIA at 340 nm over a concentration range of 1–10 μg/spot, with mean percentage recovery 100.24 ± 1.412. The selectivity of the proposed methods was tested using laboratory-prepared mixtures. The proposed methods have been successfully applied to the analysis of DIA in pharmaceutical dosage forms without interference from other dosage form additives and the results were statistically compared with reference method.

Keywords: diacerein; rhein; derivative spectrophotometry; spectrodensitometry; stability-indicating method

Introduction

Diacerein and its active metabolite rhein[1] are anthraquinone compounds that have been used in the treatment of osteoarthritis.[2–4] They act by inhibition of interleukin-1 (IL-1) synthesis, the main cytokine involved in cartilage destruction, and activation of the synthesis of proteoglycans and hyaluronic acid, the principal components of cartilage.[5] Diacerein is also used to treat and prevent vascular diseases;[6] it can be readily synthesized in few synthetic steps from the naturally occurring glucopyranoside aloin.[7,8]

In spite of its longstanding commercial distribution in oral capsule form, no official methods are available for DIA in bulk or in its pharmaceutical forms and only three analytical techniques have been reported in the literature for the quantitative determination of DIA. These are stability-indicating HPLC methods,[9,10] and a direct spectrophotometric method for determination of DIA in capsules using 0.1 N NaOH.[11]

In modern analytical laboratory, there is always a need for significant stability-indicating methods of analysis.[12,13] An ideal stability-indicating method quantifies a drug and resolves its degradation products.[14] Reviewing the literature in hand revealed that no spectrophotometric methods concerned with the determination of DIA in the presence of its alkaline degradate were reported. No synthetic mixtures were prepared to check the specificity of the reported stability-indicating HPLC methods. The aim of the present work was to develop and validate stability-indicating methods for the determinations of DIA in the presence of its alkaline degradate for the quality control and routine analysis of DIA in pharmaceutical preparations. These methods include first-derivative (D_1), first-derivative of the ratio spectra (DD_1), and TLC-densitometry.

Experimental

Apparatus

Spectrophotometer: SHIMADZU dual beam (Kyoto/Japan) UV-visible spectrophotometer model UV-1601 PC. TLC plates-precoated with silica gel F_{254}, 0.25 mm thickness (E.Merck, Darmstadt, Germany). TLC scanner 3 densitometer model 3 S/N 130319(CAMAG, Muttenz, Switzerland). Camag Linomat 5 autosampler with Camag microsyringe (100 μl); (CAMAG, Muttenz,

* *Correspondence to: Mohamed K. Abd El-Rahman, Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr-El Aini Street, 11562 Cairo, Egypt. E-mail: khaled20m20@hotmail.com*

a *Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr-El Aini street, 11562 Cairo, Egypt*

b *Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt*

Switzerland). Camag TLC scanner- Model 3 S/N 130319 with win-Cats software (CAMAG, Muttenz, Switzerland).

Reference samples

Diacerein reference standard; kindly supplied by Novartis pharm Co. (Basle, Switzerland), its purity was certified to be 99.69 ± 0.871 when assayed by the manufacturer method; (HPLC method using C_{18} column, dilute glacial acetic acid, methanol and acetonitrile (40 : 45 : 15, v/v/v) as a mobile phase and UVdetection at 254 nm).

Pharmaceutical formulation

Osteocerein® capsules manufactured by Novartis Company (Basle, Switzerland). Batch No. Y0016 and Y0028 labelled to contain 50 mg of diacerein per capsule.

Degraded samples

10 mL of 0.01M NaOH solution was added to pure DIA (500 mg) in a 100-ml round-bottom flask and left at room temperature for 5 min. Complete degradation was tested for by TLC using ethyl acetate : methanol : chloroform (8 : 1.5 : 0.5 v : v : v) as the mobile phase. Only one spot was observed not corresponding to DIA. Subsequently, 0.01M HCl solution was added to the degraded solution till pH was adjusted to about 4 and complete precipitation occurred. The precipitate was filtered and left to dry. The structure of the isolated degradation product was elucidated using mass spectrometry.

Materials and reagents

All chemicals used throughout this work were of analytical grade, and the solvents were of spectroscopic grade. Ethyl acetate, methanol, chloroform-Prolabo (VWR International, West Chester, PA, USA). DMSO; Sigma (St Louis, MO, USA).

Standard solutions

DIA stock standard solution – 0.1 mg/mL and 1 mg/mL, for the spectrophotometric and TLC-densitometric methods, respectively, were prepared in 100-mL volumetric flasks by dissolving 10, 100 mg of pure diacerein in 10 mL of DMSO and then diluting to volume with methanol. Degradation product stock solution (0.1 mg/mL) in methanol, for the spectrophotometric methods, and 1 mg/mL in methanol, for the TLC-densitometric method.

Procedures

Construction of calibration graphs

D_1 method. Aliquots (0.4–4.0 mL) of DIA stock solution (0.1 mg/mL) were transferred into a series of 10-ml volumetric flasks, and the volume was completed with methanol. The zero order spectra were recorded using methanol as a blank. The first derivative of the obtained spectra was recorded using $\Delta\lambda = 5$ nm and scaling factor 10. The peak amplitudes of the obtained first derivative spectra were measured at 322 nm. Calibration graph relating the peak amplitude to the corresponding concentrations of DIA was constructed, and a corresponding regression equation was computed.

DD_1 method. Aliquots (0.4–4.0 mL) of DIA stock solution (0.1 mg/mL) were transferred into a series of 10-ml volumetric flasks, and the volume was completed with methanol. The zero order spectra of the prepared solutions were divided by the spectrum of 4 μg/ml degradation product, the first derivative of the ratio spectra (DD_1) were obtained using scaling factor 10 and $\Delta\lambda = 5$ nm. The peak amplitudes of the first derivative of the ratio spectra were measured at 352 nm. Calibration graph relating the peak amplitudes of ($^1DD_{352}$) to the corresponding concentrations of DIA was constructed, and the corresponding regression equation was computed.

TLC-Densitometric method. Aliquots (1–10 μL) from DIA stock solution (1.0 mg/mL) were spotted onto a TLC plate using Camag Linomat autosampler with Camage micro syringe (100 μL). Spots were spaced 18.8 mm apart from each other and 15 mm from the bottom edge of the plate with a band length of 2 mm. The plates were developed in a chromatographic tanks previously saturated with the mobile phase ethyl acetate : methanol : chloroform (8 : 1.5 : 0.5 v : v : v), by ascending chromatography. The plates were dried, spots were detected under the UV lamp (254 nm), and the plates were scanned at 340 nm. A calibration graph relating the peak to the corresponding concentration of DIA was constructed, and the regression equation was computed.

Analysis of artificial mixtures

Laboratory-prepared mixtures containing DIA and different percentages of its degradation product were prepared (Tables 2, 3) and analyzed by the proposed methods.

Application of the proposed methods to the analysis of DIA in pharmaceutical preparation

D_1 and DD_1 methods. The contents of 10 capsules were mixed and weighed. A suitable portion of powder equivalent to 10 mg DIA was transferred into a 250-ml beaker, 50 ml DMSO was added and stirred for 10 min using a magnetic stirrer then filtered into a 100-mL volumetric flask and volume was completed with methanol. 2 ml of the filtrate was accurately transferred to a 10-ml volumetric flask, completed to mark with methanol. Then the procedure was completed as described earlier for construction of calibration graphs.

TLC-Densitometric method. The contents of 10 capsules were mixed and weighed. A suitable portion of powder equivalent to 25 mg DIA was transferred into a 50-ml beaker, 10 ml DMSO was added and stirred for 10 min using a magnetic stirrer then filtered into a 25-mL volumetric flask, the residue was washed 2 × 5 ml DMSO and then the volume was completed with methanol. 4.0 μL of the prepared solution was spotted in triplicate using Linomat applicator onto a TLC plate, and the procedure was completed as described earlier for construction of calibration graphs.

Results and Discussion

Diacerein is metabolized extensively (100%) in liver following oral dosing, to the deacetylated active metabolite rhein, prior to entering systemic circulation.[15] This deacetylated degradate was also obtained upon leaving DIA with alkali (Figure 1), therefore the

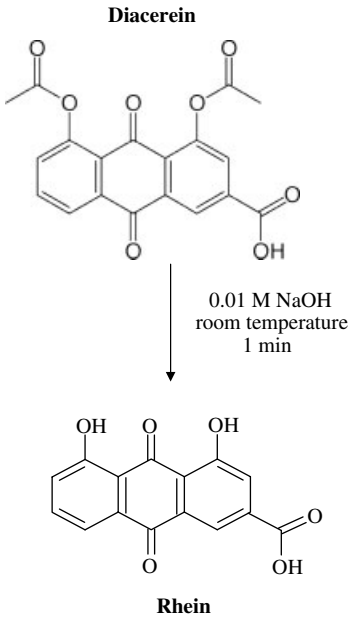

Figure 1. Alkaline degradation of DIA.[9].

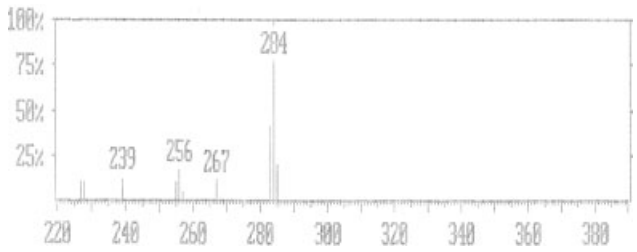

Figure 2. Mass spectra of diacerein alkaline degradate (rhein).

determination of DIA in the presence of its alkaline degradate was essential.

The structure of the alkaline degradate was elucidated by mass spectrometry (Figure 2), the electron-ionization showed mass ion peak at *m/z* 284 corresponding to the alkaline degradate which confirmed the previously reported mechanism of degradation.[9]

The focus of the present work was to develop accurate, specific, reproducible, and sensitive stability-indicating methods for the determination of DIA in pure form or in pharmaceutical formulations in the presence of its alkaline degradation product.

Table 1. Assay validation sheet of the proposed methods for the determination of pure samples of DIA and parameters of the corresponding regression equations

Parameter	DIA		
	D_1 method	DD_1 method	TLC-Densitometric method
Accuracy (mean ± SD)	100.21 ± 0.833	100.09 ± 0.912	100.24 ± 1.412
Precision			
Repeatability[a]	100.16 ± 0.988	99.82 ± 0.939	100.79 ± 0.789
Intermediate precision[b]	99.71 ± 0.781	100.48 ± 0.911	98.87 ± 0.987
Specificity	99.97 ± 1.321	100.05 ± 1.162	99.50 ± 1.287
Linearity			
Slope	0.006	−1.2464	0.0175
Intercept	0.0413	0.0369	0.1233
Corr. coeff. (r)	0.9998	0.9999	0.9997
Range	4–40 µg/mL	4–40 µg/mL	1–10 µg/spot

[a] The intra-day and [b] the inter-day mean values ± standard deviations of samples of concentration of 8, 12, 16 µg/ml of DIA by the proposed spectrophotometric methods and (4,6,8 µg/spot) for the TLC-densitometric method.

Table 2. Determination of DIA in laboratory-prepared mixtures by the proposed D_1 and DD_1 methods methods

Degradation product %	Concentration (µg/ml)		D_1 method	DD_1 method
10	36.00	4.00	101.55	100.77
20	32.00	8.00	98.65	99.19
30	28.00	12.00	101.28	99.78
40	24.00	16.00	100.88	101.71
50	20.00	20.00	98.54	98.66
60	16.00	24.00	100.21	99.33
70	12.00	28.00	98.71	98.72
80	8.00	32.0	119.33*	100.93
90	4.00	36.00	127.47*	101.36
Mean			**99.97**	**100.05**
SD			**1.321**	**1.162**
RSD%			**1.322**	**1.161**

* Rejected values.

D_1 and DD_1 methods

The zero-order absorption spectra of DIA and its alkaline degradate showed that DIA alkaline degradate overlaps with that of intact DIA and hinders direct spectrophotometric measurements (Figure 3). Derivative spectrophotometry is an analytical technique of great utility for extracting both qualitative and quantitative information from spectra composed of unresolved bands, and for eliminating the effect of baseline shifts and baseline tilts by using the first or higher derivatives of absorbance with respect to wavelength.[16] A rapid, simple, low-cost spectrophotometric method based on measuring the peak amplitude of D_1 spectrum of DIA at 322 nm (corresponding to zero-crossing of the degradate) was developed with good selectivity without interference of alkaline degradate as shown in Figure 4. In order to optimize D_1 method, different smoothing and scaling factors were tested, where a smoothing factor $\Delta\lambda = 5$ and a scaling factor = 10 showed a suitable signal to noise ratio and the spectra showed good resolution (Figure 5).

In order to improve the selectivity of the analysis of DIA in the presence of its alkaline degradate, DD_1 spectrophotometric method was also established. The main advantage of the method is that the whole spectrum of interfering substance is cancelled. Accordingly, the choice of the wavelength used for calibration is not critical as in the D_1 method.

In order to optimize DD_1 method, several divisor concentrations 4, 8, 12 and 16 µg/mL of the degradate were tried, the best result was obtained when using 4 µg/mL of the degradate as a divisor. Different smoothing and scaling factors were tested, where a smoothing factor $\Delta\lambda = 5$ and a scaling factor = 10 were

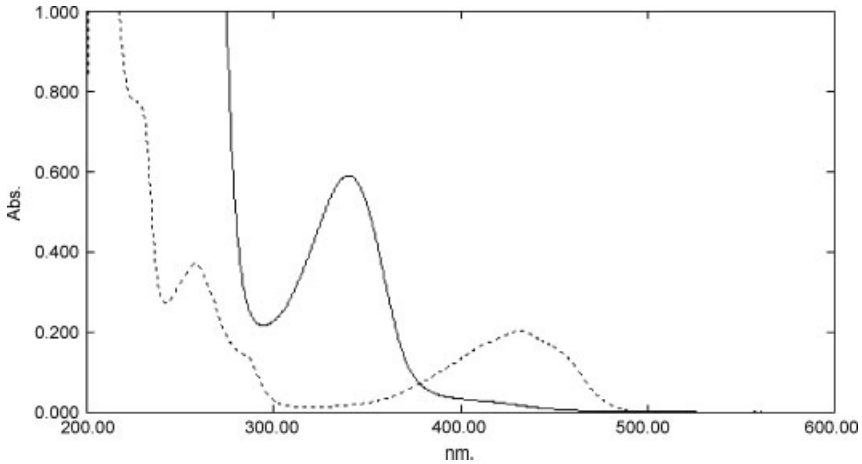

Figure 3. Zero order absorption spectra of diacerein 20 µg/mL (———) and its degradation product 4 µg/mL (· · · ·) using methanol as a solvent.

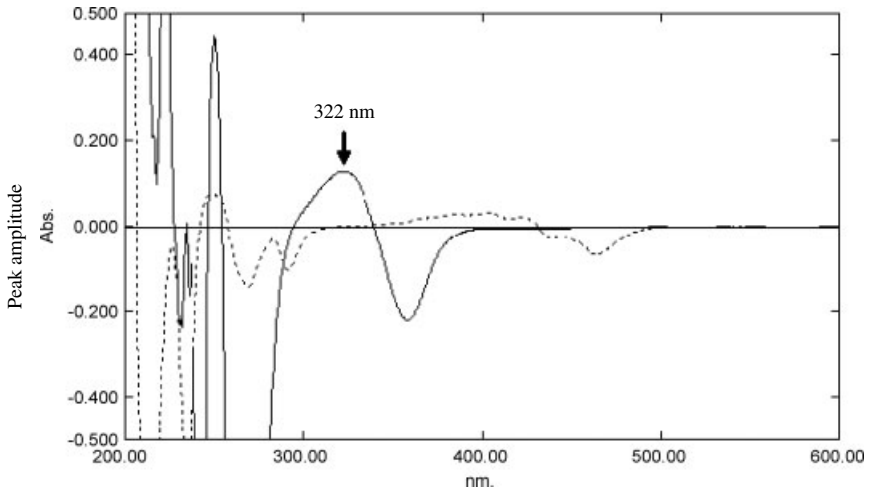

Figure 4. First-derivative absorption spectra of diacerein 20 µg/mL (———) and degradation product 4 µg/mL (· · · ·) using methanol as a solvent.

suitable to enlarge the signal of DIA to facilitate its measurement and to diminish error in reading the signal (Figures 6 and 7). The absorption spectra of DIA in the range of 4–40 µg/mL were divided by the absorption spectrum of 4 µg/mL of the degradate (as a divisor); the obtained ratio spectra were differentiated with respect to wavelength. DD_1 values showed good linearity and reproducibility at 352 nm.

The linearity of the peak amplitudes of the D_1 curves at 322 nm and the peak amplitudes of the DD_1 curves at 352 nm was studied. A linear relationship was obtained in the range from

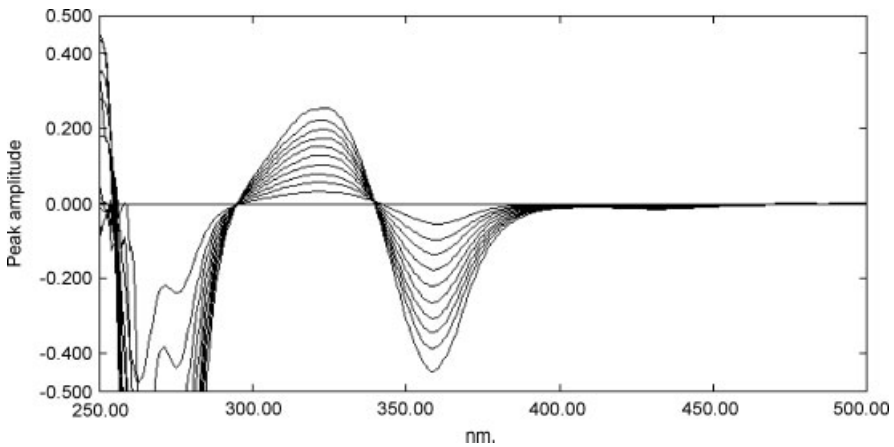

Figure 5. First-derivative absorption spectra of 4–40 µg/mL DIA.

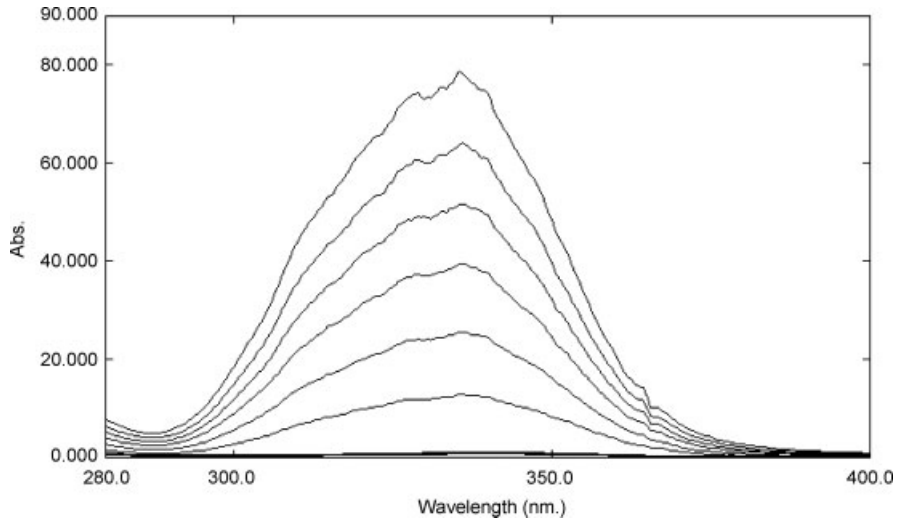

Figure 6. Ratio spectra of DIA 4–40 µg/mlL using the spectrum of 4 µg/mLof degradation product as a divisor.

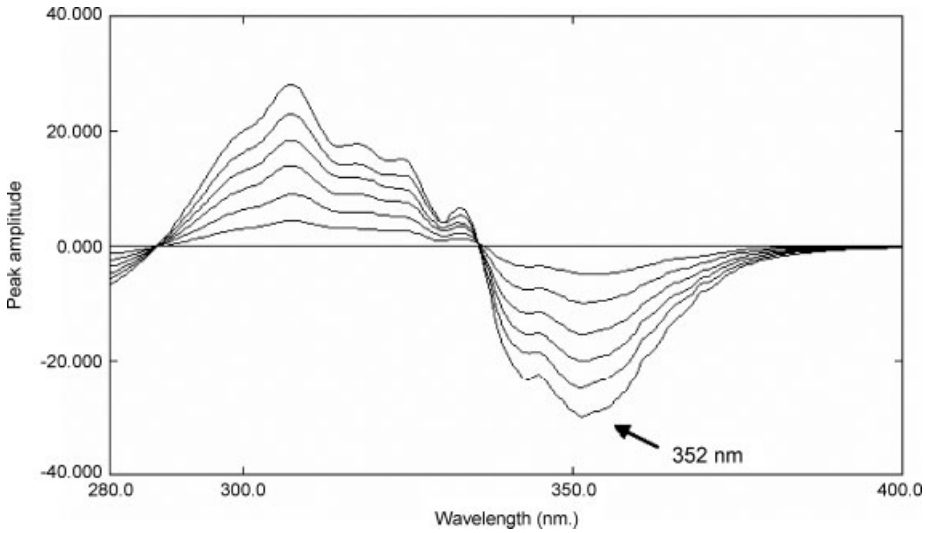

Figure 7. First derivative of ratio spectra of diacerein (4–40 µg/mL) using the spectrum of 4 µg/mL of degradation product as a divisor.

Table 3. Determination of DIA in laboratory-prepared mixtures by the proposed TLC-densitometric method

	Concentration (µg/spot)		
Mixture number	Intact	Degradation product	Recovery %
1	9.00	1.00	99.56
2	7.00	3.00	98.90
3	5.00	5.00	101.23
4	3.00	7.00	101.13
5	1.00	9.00	98.02
Mean			**99.50**
SD			**1.287**
RSD%			**1.293**

4–40 µg/mL for the drug, and the regression equations were computed (Table 1). The methods were checked by analysis of laboratory prepared mixtures of DIA and its alkaline degradate in different ratios as presented in Table 2. DIA could be determined in the presence of up to 70% of its alkaline degradate, with mean percentage recovery of 100.21 ± 0.833 in case of D_1 method. While in case of DD_1 method DIA could be determined in the presence of up to 90% of degradate with mean percentage recovery of 100.09 ± 0.912.

TLC-Densitometric method

A stability-indicating TLC-densitometric method for the determination of DIA was also described. Several trials were done to choose a developing system which can separate DIA from its degradation product including methanol : ethyl acetate : butanone (6 : 3 : 1 v : v : v) and ethyl acetate : methanol : chloroform (8 : 1.5 : 0.5 v : v : v). The first system was not satisfactory because it did not affect good separation of the drug and its degradation product; it separated the two spots but with R_f close to each other (R_f = 0.41, 0.35 for intact drug and degradation product, respectively). Satisfactory separation was obtained using the second system, ethyl acetate : methanol : chloroform (8 : 1.5 : 0.5 v : v : v) as the mobile phase. Respective R_f values were 0.35 ± 0.02, 0.61 ± 0.02 for

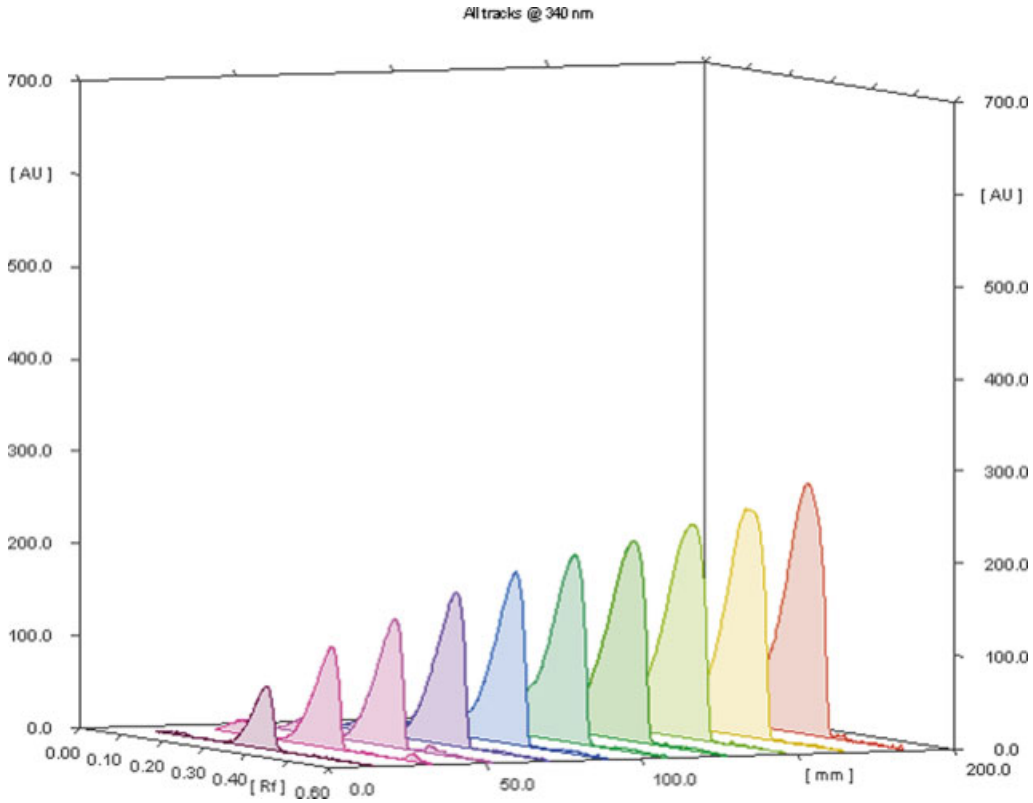

Figure 8. Scanning profile of the TLC chromatogram of DIA (1–10 µg/spot) at 340 nm.

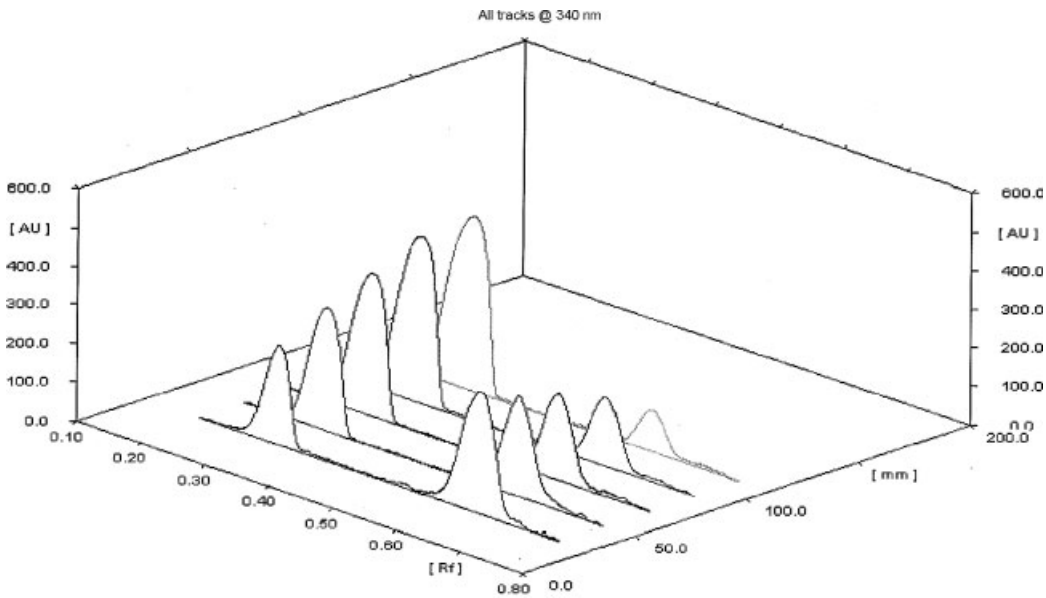

Figure 9. Thin layer chromatogram of standard DIA (1.0–9.0 µg/spot) $R_f = 0.35 \pm 0.05$ and its degradation product (9.0–1.0 µg/spot) $R_f = 0.61 \pm 0.05$ using ethyl acetate : methanol : chloroform (8 : 1.5 : 0.5 v : v : v) as a developing system.

DIA and its degradate, respectively, as shown in Figure 8. This separation allowed the determination of DIA at 340 nm without any interference from the degradation product as shown in Figure 9.

A linear relationship was found to exist between the integrated area under the peak of the separated spots at the selected wavelength (340 nm) and the corresponding concentration of DIA in the range of (1–10 µg/spot. The regression equation was computed (Table 1). The proposed method was valid for the determination of DIA in different laboratory-prepared mixtures in the presence of up to 90% of its alkaline degradate (Table 3), with mean percentage recovery of 100.24 ± 1.412.

The usefulness of the proposed methods was successfully applied for the analysis DIA in its pharmaceutical formulation and in the presence of excipients and additives was studied by

Table 4. Quantitative determination of DIA in Osteocerein® capsules by the proposed and reference methods and results of application of standard addition technique

	DIA			
	D_1 method	DD_1 method	TLC-Densitometric method	Reference method[a]
Osteocerein® capsules	%Found ± S.D.[b]			
Batch No. Y0016	99.11 ± 0.912	99.05 ± 0.888	99.71 ± 1.125	100.87 ± 0.876
Recovery of standard added %	99.66 ± 1.129	100.01 ± 0.570	100.46 ± 1.584	
Batch No. Y0028	98.19 ± 0.982	99.12 ± 0.878	100.31 ± 1.003	100.48 ± 0.924
Recovery of standard added %	99.75 ± 0.793	99.51 ± 1.006	101.33 ± 0.490	

[a] HPLC method using C_{18} column, 0.1Mphosphoric acid and methanol (40 : 60, v/v) as a mobile phase and UV detection at 254 nm
[b] Average of three determinations.

Table 5. Statistical analysis of the results obtained by the proposed methods and the reference method[13] for the determination of DIA in pure powder form

	DIA			
Item	D_1 method	DD_1 method	TLC-Densitometric method	Reference method[b]
Mean	100.21	99.88	100.24	100.09
S.D.	0.833	0.947	1.412	0.912
Variance	0.634	0.837	1.933	0.832
n	10	10	10	5
Student's t-test[b]	1.511 (2.160)	2.037 (2.160)	0.954 (2.160)	
F value[b]	1.31 (3.63)	1.01(3.63)	2.232 (3.63)	

[a] HPLC method using C_{18} column, 0.1Mphosphoric acid and methanol (40 : 60, v/v) as a mobile phase and UV detection at 254 nm
[b] Figures between parentheses represent the corresponding tabulated values of t and F at $P = 0.05$.

assaying different batches of Osteocerein® capsules. The validity of the methods was assessed by applying the standard addition technique (Table 4).

Results obtained by the proposed procedures for the determination of pure samples of the drug were statistically compared to those obtained by the reference method[9] of the drug and no significant difference was observed (Table 5). Method validation was performed according to USP guidelines[17] for all the proposed methods. Table 1 shows results of accuracy, repeatability, and intermediate precision of the methods.

Conclusion

In the present work, three simple, sensitive, rapid methods are described for determination of DIA in pure form or in pharmaceutical formulations. The proposed D_1 and DD_1 spectrophotometric methods are simple, more convenient, less time-consuming and more economic stability-indicating methods compared to other published methods. The DD_1 method has the advantage of being applicable in the presence of up to 90% of rhein. The advantages of TLC-densitometric method is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis and providing high sensitivity and selectivity. High values of correlation coefficients and small values of intercepts validated the linearity of the calibration graphs and the validity of Beer's Law. The RSD values, the slopes and the intercepts of the calibration graphs indicated the high reproducibility of the proposed methods. From the results obtained, we concluded that the suggested methods showed high sensitivity, accuracy, reproducibility, and specificity and can be used as stability-indicating methods. Moreover, these methods are simple and inexpensive, permitting their application in quality control laboratories.

References

[1] T. Tamura, K. Ohmori, *Jpn. J. Pharmacol.* **2001**, *85*, 101.
[2] E. Douni, P. P. Sfikakis, S. Haralambous, P. Fernandes, G. Kollias, *Arthritis Res. Ther.* **2004**, *6*, R65.
[3] J. P. Pelletier, D. Lajeunesse, P. Reboul, F. Mineau, J. C. Fernandes, P. Sabouret, J. Martel-Pelletier, *J. Rheumatol.* **2001**, *28*, 814.
[4] G. N. Smith Jr., S. L. Myers, K. D. Brandt, E. A. Mickler, M. E. Albrecht, *Arthritis Rheum.* **1999**, *42*, 545.
[5] M. Solignac, *Presse Med.* **2004**, *33*, S10.
[6] T. Cruz, A. Pastrak, *PCT Int. Appl.* **2003**, pp. 26.
[7] M. Sinistri, R. Sinistri, *PCT Int. Appl.* **1998**, pp. 12.
[8] D. Maggi, *PCT Int. Appl.* **2004**, pp. 10.
[9] V. Giannellini, F. Salvatore, G. Bartolucci, S. A. Coran, M. B. Alberti, *J. Pharm. Biomed. Anal.* **2005**, *39*, 776.
[10] J. Rao, K. Chauhan, K. R. Mahadik, S. S. Kadam, *Indian J. Pharm. Sci.* **2009**, *71*.
[11] S. H. M. Borgmann, L. M. Parcianello, M. Z. Arend, S. G. Cardoso, *Pharmazie* **2007**, *62*, 483.
[12] M. A. El-Sayed, *Drug Test. Anal.* **2009**, *1*, 279.
[13] M. A. El-Sayed, M. A. Mohammad, *Drug Test. Anal.* **2009**, *1*, 228.
[14] A. Ashour, M. A. Hegazy, A. A Moustafa, K. O. Kelani, L. E. Abdel Fattah, *Drug Test. Anal.* **2009**, *1*, 327.
[15] *Martindale, 'The Extra Pharmacopoeia', The Complete Drug Reference, 34th edn*, The Pharmaceutical Press, London, **2005**.
[16] J. J. Berzas Nevado, J. Rodriguez Flores, M. J. Villasenor Lierena, *Anal. Lett.* **1994**, *27*, 1009.
[17] The United States Pharmacopeia and National Formulary, *The Official Compendia of Standards, Asian Edition, USP 30-NF 25*, The United States Pharmacopeial Convention Inc.: Rockville, Maryland, **2007**.