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Quantitative analysis of anti-inflammatory drugs using FTIR-ATR spectrometry

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ABSTRACT

Four simple, accurate, sensitive and economic Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopic (ATR-FTIR) methods have been developed for the quantitative estimation of some non-steroidal anti-inflammatory drugs. The first method involves the determination of Etodolac by direct measurement of the absorbance at 1716 cm^{-1}. In the second method, the second derivative of the IR spectra of Tolfenamic acid and its reported degradation product (2-chlorobenzoic acid) was used and the amplitudes were measured at 1084.27 cm^{-1} and 1056.02 cm^{-1} for Tolfenamic acid and 2-chlorobenzoic acid, respectively. The third method used the first derivative of the IR spectra of Bumadizone and its reported degradation product, *N,N*-diphenylhydrazine and the amplitudes were measured at 2874.98 cm^{-1} and 2160.32 cm^{-1} for Bumadizone and *N,N*-diphenylhydrazine, respectively. The fourth method depends on measuring the amplitude of Diacerein at 1059.18 cm^{-1} and of rhein, its reported degradation product, at 1079.32 cm^{-1} in their first derivative spectra. The four methods were successfully applied on the pharmaceutical formulations by extracting the active constituent in chloroform and the extract was directly measured in liquid phase mode using a specific cell. Moreover, validation of these methods was carried out following International Conference of Harmonisation (ICH) guidelines.

1. Introduction

Fourier Transform-Infrared (FT-IR) spectroscopic analytical methods are convenient, rapid, and accurate methods, especially when in conjunction with Attenuated Total Reflectance (ATR) technology. ATR is a leading FT-IR sampling tool which typically eliminates sample preparation thus saving considerable analysis time due to its ease-of-use and speed of analysis [1]. Selection of the best; ATR crystal and accessory; further enhances FT-IR sampling success and simplifies sample handling [2]. Moreover, ATR technique provides a simple approach that control many sample handling problems and explores the mid-IR spectroscopy as an analytical technique in a number of areas, including pharmaceutical samples [3].

Etodolac (ET) is chemically known as 2-(1,8-diethyl-4,9-dihydro-3H-pyrano[3,4-*b*]indol-1-yl)acetic acid (Fig. 1A) [4]. ET works by reducing the levels of prostaglandins responsible for pain, fever and tenderness that occur with inflammation. It blocks the enzyme that is responsible for the synthesis of prostaglandins (cyclooxygenase) thereby resulting into lower concentrations of prostaglandins. Consequently, inflammation, pain and fever are reduced [5,6]. Various methods have been reported for the determination of ET in drug formulations and in biological samples. Reported methods for the determination of ET are based on spectrophotometry [7], high performance liquid chromatography (HPLC) with UV detection [8] and HPLC with mass spectrometric detection [9].

Tolfenamic acid (TA), is chemically known as 2-(3-chloro-2-methylanilino)benzoic acid (Fig. 1B) [4]. TA is a non-steroidal anti-inflammatory (NSAID) drug that belongs to the family of fenamates and is used in both humans and animals for the management of pain and inflammation [10]; 2-chlorobenzoic acid (2CHB) one of TA degradation products (Fig. 1C) [9]. Recently, TA has gained tremendous popularity due to its anticancer activity against a variety of cancers [11]. It has also shown potential for use in slowing down the progression of Alzheimer's disease [12]. A quantitative analysis of Tolfenamic Acid (TA) both as a pure compound and in tablet dosage form has been carried out using FT-IR and UV spectroscopy [13].

Bumadizone (BUM) chemically known as 2-[anilino(phenyl)carbamoyl] hexanoic acid (Fig. 1D) [4], is a non-steroidal anti-inflammatory drug; its oral preparation has been used for the treatment of rheumatic disorders and post-traumatic edema [14]. Few methods have been reported for its estimation. These methods include determination of BUM and its two reported metabolites, phenylbutazone

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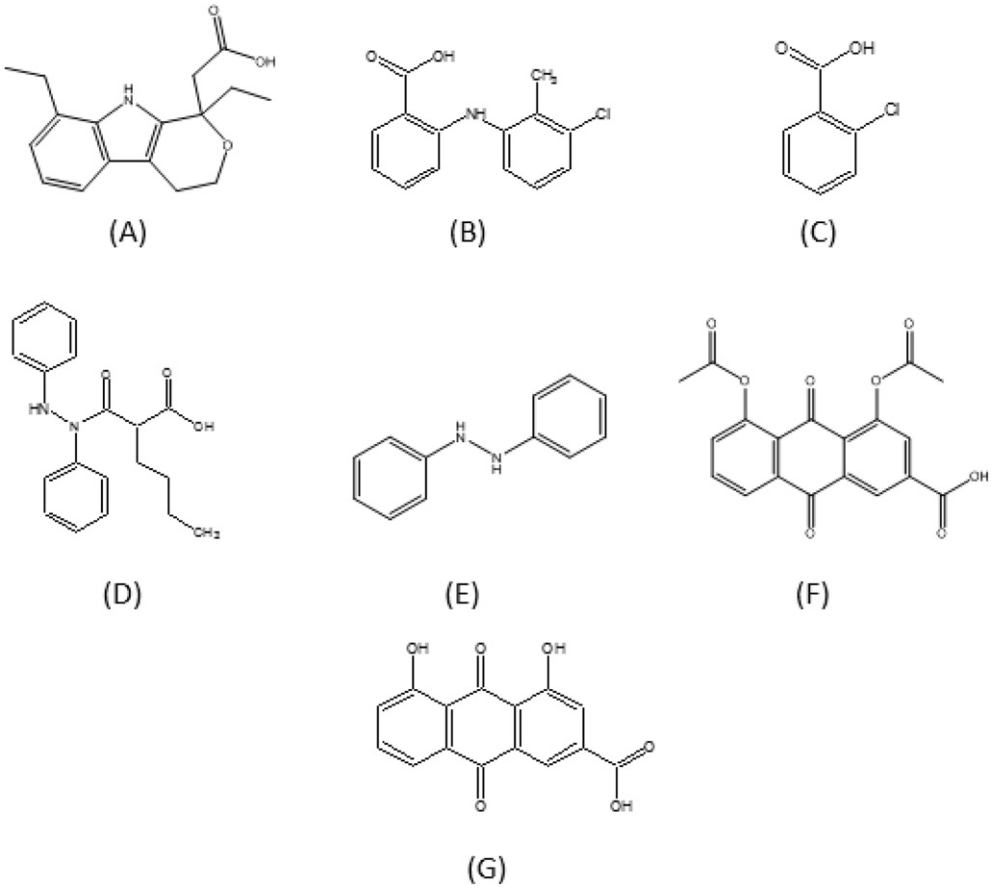


Fig. 1. Chemical structure of (A) Etodolac, (B) Tolfenamic acid, (C) 2-chlorobenzoic acid, (D) Bumadizone, (E) *N,N*-diphenylhydrazine, (F) Diacerein and (G) Rhein.

and oxyphenbutazone, by HPLC [15]. A stability indicating HPLC-UV method is also reported for the determination of BUM in the presence of its alkaline degradation product, N—N diphenylhydrazine (PH) (Fig. 1E), which was prepared by refluxing with 1 M NaOH for 7 h [16].

Diacerein (DIA) is chemically known as 4,5-diacetyloxy-9,10-dioxoanthracene-2-carboxylic acid (Fig. 1F) [10]. It acts by inhibiting interleukin-1 synthesis [17] and used in the treatment of osteoarthritis. Diacerein rapidly metabolizes in the body to the active metabolite, rhein (Fig. 1G) [18], which is used as the starting material for the synthesis of Diacerein [19] and also considered as impurity [10].

The aim of this research work is to develop an alternative method for the routine quantification of some anti-inflammatory drugs in the presence of their degradation products. Moreover, derivative spectroscopy was used for data processing to overcome the spectral overlap to allow simultaneous estimation of each drug and its degradation product.

2. Experimental Section

2.1. Instrumentation

The IRAffinity-1 Fourier Transform Infrared Spectrophotometer (Schimadzu Corporation, Tokyo, Japan) was connected to an ATR-8200H/8200HA base unit. A demountable liquid transmission cell (Wilmad Lab glass, Buena, NJ, USA) with ZnSe trough plate 45° prism (wave number range 10,000–55 cm^{-1}, refractive index 2.4, 1 mm thick, and 0.05 mm optical path-length Transmission Range (700–4600 cm^{-1})) was used. An automatic pipette (10–100 μg) was used for carrying either sample solution or standard solution into the flow cell.

2.2. Reagents and Reference Samples

Chloroform (HPLC grade, for liquid sample preparation) was supplied by Sigma-Aldrich, Germany.

Etodolac (99.13 ± 0.729, [20]) was supplied by European Egyptian Pharmaceutical Industries, Alexandria, Egypt and Etodolac® tablets (Batch No. 4015001) labeled to contain 300 mg Etodolac per tablet were purchased from market.

Tolfenamic acid (99.70 ± 1.098, [13]) was supplied by Galen Limited, UK and Clotam® Rapid tablets (Batch No. 31022276) labeled to contain 200 mg Tolfenamic acid each were purchased from market.

Bumadizone (99.70 ± 1.14, [16]) was supplied by October pharma S.A.E., 6 October City, Egypt and Octomotol® tablets (Batch No. B03660613) labeled to contain 110 mg Bumadizone per tablet were purchased from market.

Diacerein (100.63 ± 1.266, [21]) was supplied by Eva pharma, Giza, Egypt and Diacerein® capsules (Batch No. 601306) labeled to contain 50 mg Diacerein per tablet were purchased from market.

2-Chlorobenzoic acid was purchased from VEB-Laborchemie Apolda, Germany. N—N diphenylhydrazine was purchased from Central drug house (P) Ltd. Daryagani, New Delhi, India. Rhein was purchased from Ningbo ETDZ Conner Pharmatech Co., Beilun, Ningbo, China.

2.3. Standard and Test Solutions

2.3.1. Stock Standard Solutions

ET, DIA, RH, BUM, PH, TA and 2CHB stock solutions (0.1 mg/mL) were prepared by dissolving in chloroform. Sonication for 10 min was used to ensure complete dissolution.

2.3.2. Laboratory-prepared Mixtures

Different aliquots of TA equivalent to (150–750 μg) and 2CHB equivalent to (200–800 μg), of BUM (200-900 μg) and PH (400–900 μg) or DIA (300–1000 μg) and RH (100–800 μg) were transferred into three series of 10 mL volumetric flasks.

All the flasks were completed to volume with chloroform to obtain different laboratory prepared mixtures.

2.3.3. Sample Preparation

Twenty Etodolac tablets® (300 mg Etodolac) were weighed and finely grounded. A quantity equivalent to 10 mg ET was quantitatively transferred into 100 mL volumetric flask, 50 mL chloroform was added and the flask was sonicated for 10 min. The flask was then completed to volume with chloroform (0.1 mg/mL). The solution was filtered. Aliquots of this solution equivalent to (450–850 μg) were transferred into separate 10 mL volumetric flasks and completed to volume with chloroform.

The same extraction procedure was applied for Clotam rapid ® (200 mg Tolfenamic acid) and Octomotol® (110 mg Bumadizone) to prepare sample solution concentration (0.1 mg/mL) of each pharmaceutical formulation. Aliquots of Octomotol and Clotam extracted solution equivalent to (250–500 μg) for TA and (350–750 μg) for BUM were transferred into two sets of 10 mL volumetric flasks and completed to volume with chloroform.

Twenty Diacerein® hard gelatin capsules (50 mg Diacerein) were carefully opened and emptied in a dry clean weighing bottle. A quantity of the powder equivalent to 10 mg DIA was transferred into 100 mL volumetric flask dissolved in 50 mL chloroform and sonicated for 10 min. The solution was completed to volume with chloroform then filtered (0.1 mg/mL). Aliquots of the sample solution equivalent to (350–750 μg), were transferred into separate 10 mL volumetric flasks and completed to volume with chloroform.

2.4. Procedures and Calibration Curves

2.4.1. General Procedure

From each prepared solution, 10 μL was placed on a diamond cell ATR accessory (ZnSe) with the help of a micropipette to record the spectrum. All the spectra were collected by co-addition of 40 scans at a resolution of 5 cm^{-1} in the range of 4000–500 at 1.93 cm^{-1} data spacing. The spectrum of each standard or sample was subjected to the ratio of that to a fresh background spectrum recorded from the uncovered removable diamond crystal. All analyses were carried out at room temperature, and the spectra were each recorded and auto smoothed to remove noise. ATR crystal was carefully cleaned with a cellulose tissue soaked in acetone after each sample to remove any residue.

2.4.2. Construction of Calibration Curves

2.4.2.1. Direct Measurement of Etodolac. Aliquots of ET stock solution equivalent to 300–1000 μg were accurately transferred into a series of 10 mL volumetric flasks and the volumes were completed with chloroform. The absorbance was measured at 1716 cm^{-1} (Fig. 2).

2.4.2.2. Second Derivative ATR-FTIR Spectroscopic Measurement of Tolfenamic Acid and 2-Chlorobenzoic Acid. Aliquots of TA stock and 2CHB solution equivalent to 150–750 μg and 200–800 μg respectively, were accurately transferred into a series of 10 mL volumetric flasks and the volumes were completed with chloroform. The second derivative spectra were recorded, the trough amplitude of TA was measured at 1084.27 cm^{-1} and the peak amplitude of 2CHB was measured at 1058.02 cm^{-1} (zero crossing points) (Fig.3C).

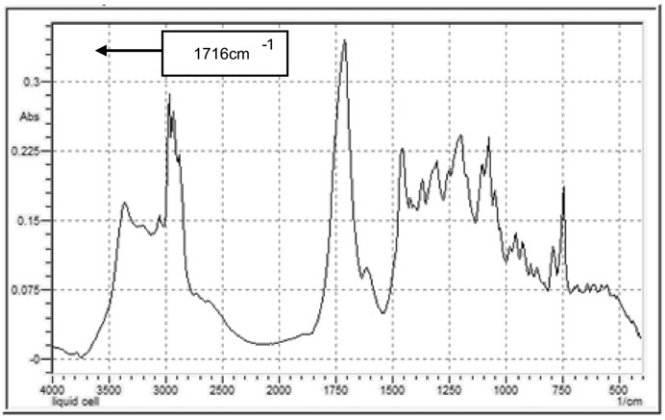


Fig. 2. ATR-FTIR spectrum of Etodolac (55 μg/mL).

2.4.2.3. First Derivative ATR-FTIR Spectroscopic Measurement of Bumadizone and N,N-Diphenylhydrazine. Aliquots of BUM and PH stock solutions, equivalent to 200–900 μg and 400–900 μg respectively, were accurately transferred into two series of 10 mL volumetric flasks and the volumes were completed with chloroform. The first derivative spectra were recorded, the trough amplitude of BUM measured at 2874.98 $cm^{-1}$and the peak amplitude of PH was measured at 2160.32 cm^{-1} (zero crossing points) (Fig.4B).

2.4.2.4. First Derivative ATR-FTIR Spectroscopic Measurement of Diacerein and Rhein. Aliquots of DIA and RH stock solutions, equivalent to 300–1000 μg and 100-800 μg respectively, were accurately transferred into two series of 10 mL volumetric flasks and the volumes were completed to the mark with chloroform. The first derivative spectra were recorded, the trough amplitude of DIA was measured at 1059.18 cm^{-1} and the peak amplitude of RH was measured at 1079.32 cm^{-1} (zero crossing points) (Fig.5B).

All the calibration curves were constructed by plotting the absorbances or amplitudes against the corresponding concentrations of each drug (μg/mL).

3. Method Validation

3.1. Linearity

Standard samples of ET, DIA, RH, BUM, PH, TA and 2CHB were prepared in different concentration range. Each concentration was analyzed three times. Good linearity of the calibration curves was verified by the high correlation coefficient. The analytical data of the calibration curve including standard deviations for the slope and intercept (S_b, S_a) are summarized in Table 1.

3.2. LOD & LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated using the following equations. LOD = 3.3 (SD/Slope) & LOQ = 10 (SD/Slope) where SD is the standard deviation of the response.

3.3. Precision

The repeatability (Intraday precision) of the method was assessed by six determinations for each of the three representing concentrations [(40–45-50), (40–45-50), (30–35-40), (35–40-45), (50–55-60), (30–35-40), (30–35-40) μg/mL] for ET, DIA, RH, BUM, PH, TA and 2CHB respectively. The repeatability were expressed in terms of percentage relative standard deviation (%R.S.D.). All experiments were repeated in three consecutive days by the same analyst to evaluate intermediate precision (Interday).

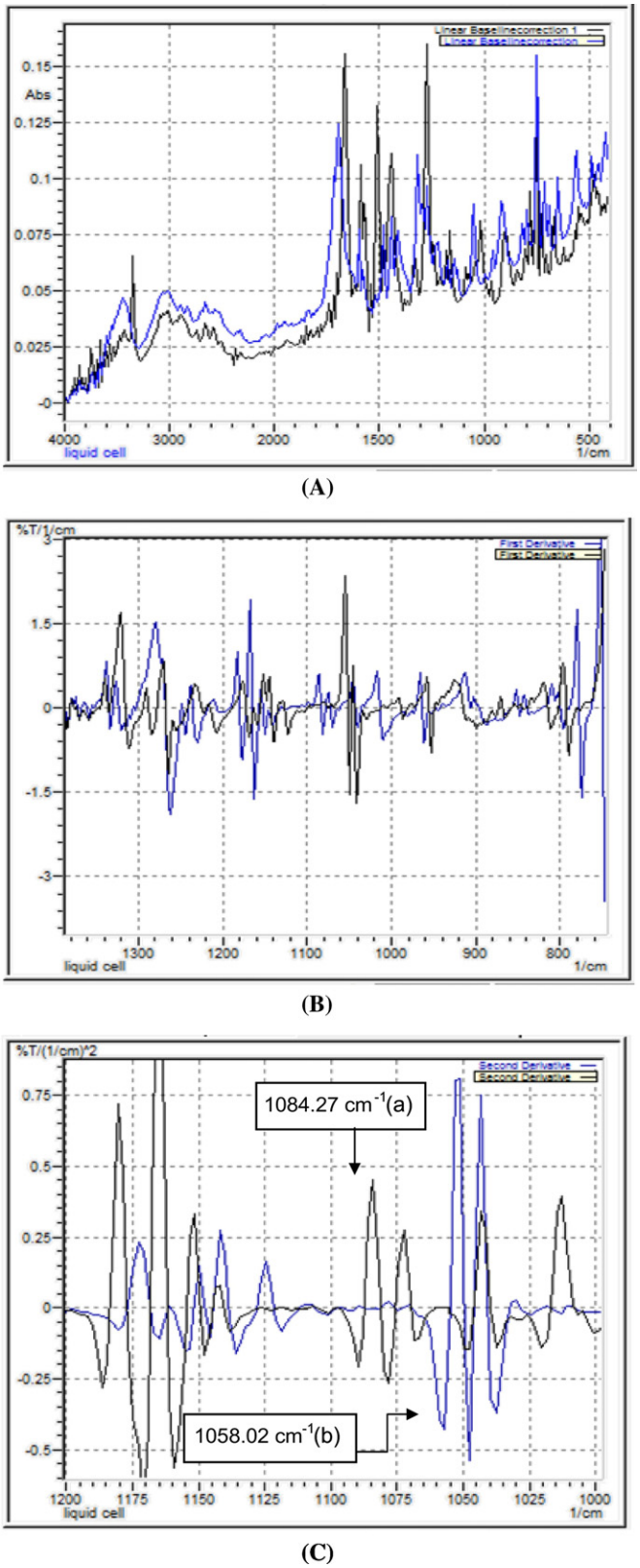


Fig. 3. **(A)** ATR-FTIR spectra, **(B)** first derivative and **(C)** second derivative ATR-FTIR spectra of (a) Tolfenamic acid (40 μg/mL) and (b) 2-chlorobenzoic acid (45 μg/mL).

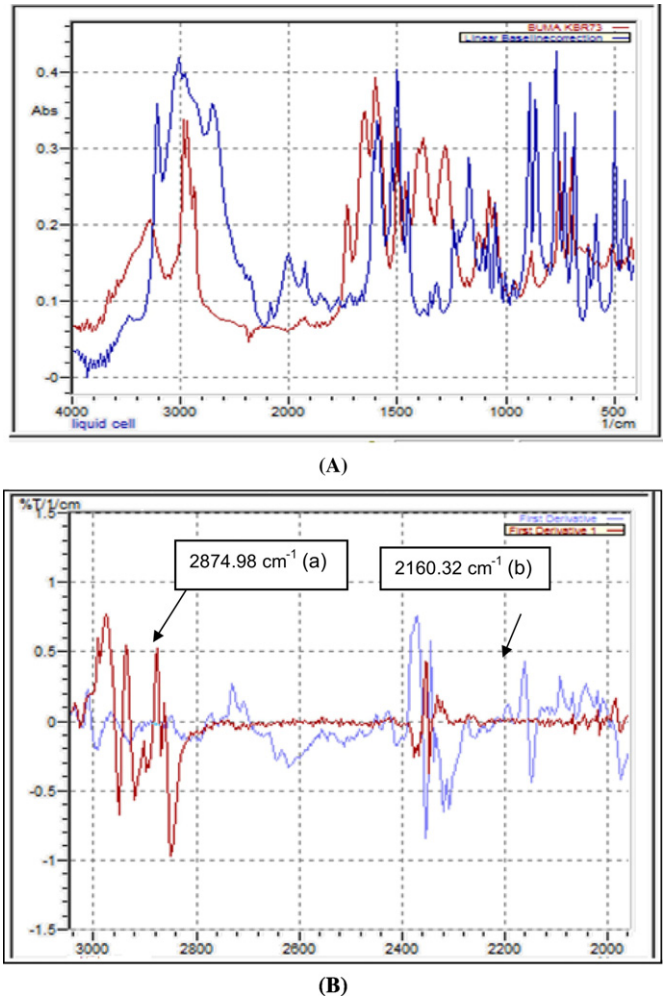


Fig. 4. **(A)** ATR-FTIR spectra and **(B)** first derivative ATR-FTIR spectra of (a) Bumadizone (57 μg/mL) and (b) *N,N*-Diphenylhydrazine (66 μg/mL).

3.4. Accuracy

Recovery studies were performed by applying the regression equations of ET, DIA, RH, BUM, PH, TA and 2CHB to determine each of them in substance. The accuracy of the methods was also confirmed by recovery studies from dosage forms at different levels of standard additions. The mean percentage recoveries are displayed in Table 1.

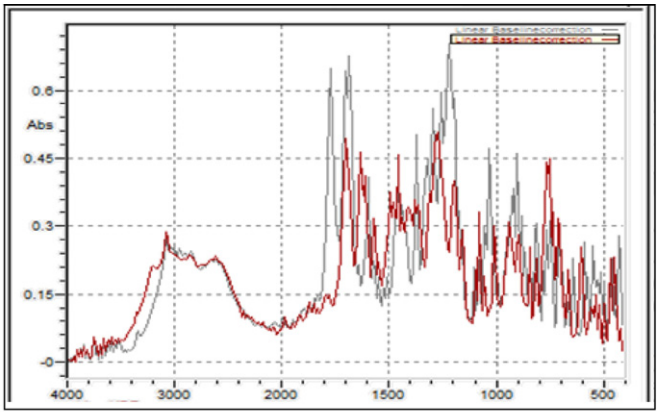
3.5. Selectivity

Three of the cited drugs were successfully determined by the presented methods in admixture with their degradation products. The results are displayed in Table 1. The four drugs were also determined in their dosage forms without any interference from the excipients and this was proven by overlaying the spectra of the authentic solutions and the pharmaceutical dosage forms extracts as shown in Fig. 6. The standard addition technique was also applied.

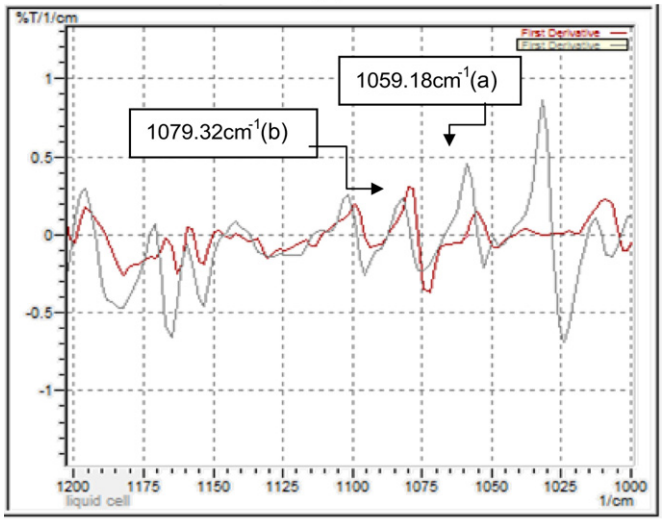
4. Results and Discussion

The main benefit of using a diamond cell ATR smart accessory is its simplicity in handling. It only requires to place the sample on the crystal and scanned against the background of the clean crystal.

ET solution was scanned and its absorbance showed high sensitivity at 1716 cm^{-1} due to carbonyl stretching, (Fig. 2) and also it showed good linearity when the absorbances were plotted against different concentrations.



(A)



(B)

Fig. 5. (A) ATR-FTIR spectra and **(B)** first derivative AT-FTIR spectrum of (a) Diacerein (52 μg/mL) and (b) Rhein (39 μg/mL).

In the second method, the zero order spectra of TA and 2CHB showed severe overlap (Fig. 3A), which prevents their direct measurement without preliminary separation. Thus, the derivative of the amplitude spectra was suggested to resolve this problem. By a thorough look in the first derivative spectra (Fig, 3B), no suitable peak for measurement of either TA or 2CHB was found while in the second derivative spectra of TA showed a peak at 1084.27 cm^{-1} where 2CHB displayed zero value and 2CHB showed a peak at 1058.02 cm^{-1} where TA displayed zero value (Fig. 3C).

The same goes to the zero order spectra of BUM and PH (Fig. 4A), which showed severe overlap that prevents their direct measurement without preliminary separation. The first derivative spectra (Fig. 4B) of BUM showed a peak at 2874.98 cm^{-1} where PH displayed zero value and PH showed a peak at 2160.32 cm^{-1} where BUM displayed zero value.

In the same vein, the zero order spectra of DIA and RH showed severe overlap (Fig. 5A). Determination of both DIA and RH were achieved using the first derivative spectra (Fig. 5B) by measuring the amplitude at 1059.18 cm^{-1} where RH displayed zero value and at 1079.32 cm^{-1} where DIA showed zero value, respectively.

The above selected wavenumbers absorbances or amplitudes were used successfully for the determination of the aforementioned drugs without any interference.

5. Conclusion

Four new precise and accurate quantitative ATR-FTIR methods with minimal sample preparation has been realized for the determination of four non-steroidal anti-inflammatory drugs, Etodolac, Tolfenamic acid, Bumadizone and Diacerein, either alone or in presence of their reported degradation products. Moreover, 2-chlorobenzoic acid, *N*,*N*-diphenylhydrazine and rhein, three reported degradation products, were successfully determined simultaneously with their intact drugs, Tolfenamic acid, Bumadizone and Diacerein, respectively. Analysis was easily carried out for these NSAIDs in their liquid form without preliminary purification. These analytical methods are alternative to those that employ separation techniques. This can significantly reduce both expensive laboratory analysis and chemical waste.

Appendix A. Supplementary Data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.saa.2017.06.002.

Table 1
Validation data for FTIR spectrometric analysis.

NSAIDs	ET	DIA	RH	BUM	PH	TA	2CHB
Wavenumber of measurement (cm^{-1})	1716.00	1059.06	1078.59	2160.32	2874.98	1084.27	1058.02
Solvent	chloroform						
Linearity Range (μg/mL)	30–100	30–100	10–80	20–90	40–90	15–75	20–80
Regression coefficient (r^2)	0.999	0.998	0.999	0.999	0.999	0.998	0.999
Slope	0.0068	0.009	0.0077	0.0089	0.0067	0.0103	0.0088
Intercept	−0.007	−0.009	0.050	0.026	0.033	0.0203	0.0580
LOD (μg/mL)	1.523	2.568	2.826	2.773	1.193	2.729	1.573
LOQ (μg/mL)	4.614	7.782	8.566	8.402	3.616	8.271	4.7670
S_b	0.003	0.008	0.005	0.007	0.004	0.008	0.004
S_a	0.0001	0.0001	0.0001	0.0001	0.0001	0.0002	0.0001
Confidence limit of the slope	0.0001	0.0002	0.0002	0.0002	0.0002	0.0004	0.0002
Confidence limit of the intercept	0.008	0.019	0.013	0.002	0.011	0.020	0.011
Standard error of the estimation	0.003	0.007	0.007	0.007	0.002	0.008	0.004
Inter-day (%R.S.D.)	0.265	0.152	0.257	0.288	0.301	0.243	0.343
Intra-day (%R.S.D.)	0.224	0.173	0.231	0.278	0.320	0.233	0.3211
Drug in bulk	99.78 ± 0.704	99.89 ± 0.314	–	99.72 ± 0.545	–	99.62 ± 0.482	–
Laboratory prepared mixture		99.66 ± 0.659	99.87 ± 0.530	100.12 ± 0.753	99.70 ± 0.539	101.48 ± 0.554	99.73 ± 0.768
Drug in dosage form	100.02 ± 0.449	99.92 ± 0.243	–	99.83 ± 0.664	–	99.23 ± 0.685	–
Drug added	99.34 ± 0.586	99.89 ± 0.633		99.62 ± 0.766		99.18 ± 0.756	

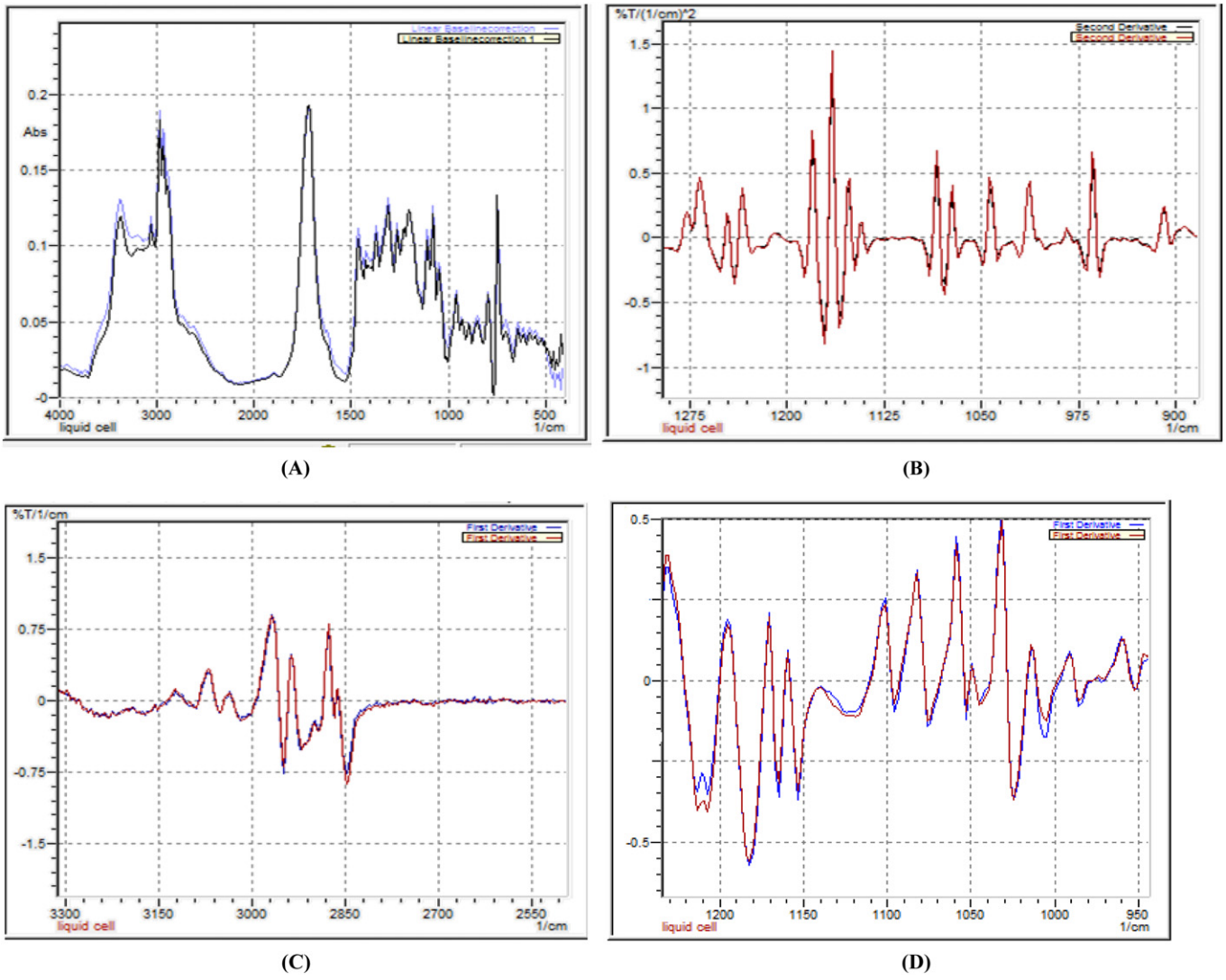


Fig. 6. **(A)** FTIR spectra of Etodolac (30 μg/mL) and Etodolac® tablets (30 μg/mL), **(B)** second derivative-FTIR spectra of Tolfenamic acid (60 μg/mL) and Clotam® tablets (60 μg/mL), first derivative-FTIR spectra of **(C)** Bumadizone (85 μg/mL) and Octomotol® tablets (85 μg/mL) and **(D)** Diacerein (45 μg/mL) and Diacerein® capsules (45 μg/mL).

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