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Validated ATR-FTIR Method For Quantitative Simultaneous Estimation of Terbutaline Sulfate, Guaiphenesin and Bromhexine Hydrochloride, in Bulk and Pharmaceutical Dosage Form

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Abstract

Using Attenuated Total Reflectance Fourier Transform Infrared Spectrometry, the current study aimed to develop and successively validate a simple, accurate method for simultaneous estimation of terbutaline sulphate, guaifenesin, and bromhexine hydrochloride in bulk, pharmaceutical preparation, or combination. Absorbance of the first derivative amplitude with a correlation coefficient (r) better than 0.99 was chosen for sample quantification after calibration models were established based on measurements of the three substances in their first derivative spectra zone at 4600-400 cm^{-1} Absorbance was determined at 1151cm^{-1}, 1332cm^{-1}, and 919 cm^{-1} for three compound respectively; after the spectra were transformed to first order derivative spectra ($\Delta\lambda$=1, scaling factor=10). This method was compared to the standard UV-visible spectrophotometry technique for quantitative sample analysis. The suggested technique shows promise as a quick screening tool due to a high correlation (r = 0.97) between the two approaches' predictions of content across samples. It was then shown that the installed method was suitable for determining the active ingredients in commercial and model cough syrup, with no interference from the formulation excipients being observed; these results suggested that this attenuated total Reflectance fourier transform Infrared could be used for the determination of the multiple active ingredients in their dosage form.

Keywords: Terbutaline sulphate; Guaifenesin; Bromhexine hydrochloride; Attenuated total reflectance Fourier transform infrared, first derivative.

1.Introduction

Attenuated Asthma, chronic bronchitis, emphysema, and other lung illnesses may cause shortness of breath and other breathing difficulties; a "reliever" inhaler containing terbutaline TBN; (figure-1) can help alleviate these symptoms [1]. It is a crystalline material that is either white or gray in color. It has no smell or just a trace of acetic acid smell [2]. 5-[2-(tert-butylamino)-1-hydroxyethyl] benzene-1,3-diol is the chemical name for this compound [3]. As shown in (figure-2), guaifenesin (GUF) is an expectorant used to facilitate the expelling of mucus from the respiratory tract by coughing. The mucus in your airways will be thinner after taking guaifenesin, making it simpler to cough up and get rid of. [1] It's a crystalline material that's white or very little gray and has a somewhat bitter aromatic flavor. [2] It is 3-(2methoxyphenoxy)-1,2-propanediol is its chemical name [3]. (figure-3) Bromhexine hydrochloride (BHN) is a mucolytic medication used to treat respiratory conditions characterized by thick, sticky mucus. [4] Formulations may include varying amounts of excipients such as a preservative, sweetener, acidulate, artificial color, or flavoring ingredient [3].

Total Reflection (ATR) spectroscopy is a universal, non-destructive analytical technique with feasible sample recovery for solid or liquid transmission analyses. Overall, Sample preparation method using ATR can be considered as time efficient compared to solid FTIR. [5] Therefore, the most commonly technique used when studying solutions is ATR-FTIR spectroscopy (figure- 4), which usually utilizes a diamond as, Ge, Si or ZnSe IRE. Recent research has identified that IgG aggregates distribute unevenly close to the surface of the ZnSe using ATR-FTIR spectroscopic imaging approach [6]. The beam

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of infrared light passing through a ZnSe crystal which allows total internal reflection [5] penetrates the surface of a liquid sample, and the eventual signal received at the detector can be used to characterize the sample transmission (figure- 4). After the method was completed, a diamond cell was then cleaned with alcohol (70% v/v) and dried, to make sure the ATR crystal was clean, a spectrum was collected using the latest background for reference. BRUKER OPUS software was used to convert the spectra to absorbance. ATR-FTIR is gaining much interest as an environmental friendly analytical technique, for qualitative and quantitative estimation of chemical substances in the of fields chemistry, pharmacy, forensics, and food analysis, among others experimental sciences., e.g. Spectroscopy Analysis of Saliva for Breast Cancer Diagnosis [6] also in rapid Non-Destructive Ink Discrimination of Forensic Documents [7] for the of biopharmaceuticals [8] quantification of water content in deep eutectic solvents, [9] and other liquids such as milk[10], honey,[11], etc. The ATR sampling allows for quick and easy analysis without the need for time-consuming sample preparation. The sample shape, the angle of incidence of the infrared beam inside the crystal, and the applied pressure are all areas where the accessory may be fine-tuned for optimal performance. [12]
FTIR is a great quantitative method since it can be used in tandem with programs like Partial Least Squares (PLS) regression to do multicomponent analysis with ease. Very high sensitivity with little sample preparation distinguishes the ATR-FTIR technique from the UV-spectrophotometric as the most valuable option. [13].

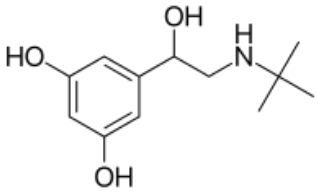

Figure-1: chemical structure of Terbutaline.

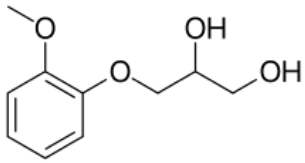

Figure-2: chemical structure of Guaifenesin.

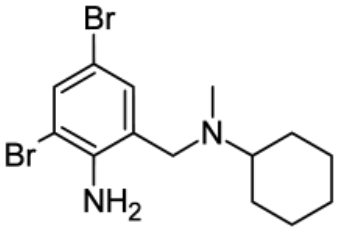

Figure-3: chemical structure of Bromhexine.

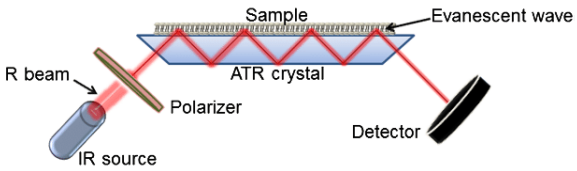

Figure-4: schematic representation of an ATR-FTIR technique.

2. Experiment

2. 1. Material and Methodology

2.1.1. Reagents and Chemicals

SEDICO Pharmaceutical Company provided terbutaline sulphate (99.70 1.14). Guaifenesin (99.90 1.11) was given by Rameda pharmaceutical firm on October 6 in Giza, Egypt. Amriya pharm industries., Alexandria, Cairo, Egypt, provided the bromhexine hydrochloride used in the experiment, which took place on October 6 at City, Giza, Egypt. Using a Milli Q water purification system, we made ultra-pure water, and cough syrup from ingredients found in Egyptian markets (Peenya, Bangalore, India).

2.1.2. Instrumentation

A Schimadzu IRAffinity-1 FT-IR Spectrometer (Tokyo, Japan) was linked to an ATR-8200H/8200HA. The spectral resolution was set at 4 cm1 using a ZnSe trough plate 45o prism in a detachable liquid transmission cell made of Wilmad Lab glass from Buena, NJ, USA (wave number range 10000-55-cm^{-1}, refractive index 2.4, 1 mm thick, and 0.05 mm optical path-length Transmission Range (400-4600cm^{-1}). The sample and/or reference solutions were pipetted into the flow cell using an automated pipette (10-100 µg). Recording spectrophotometer for UV and visible light by Schimadzu (UV-160A PC, Schimadzu, Kyoto, Japan). Sonicator (model USR3/2 907, Julabo Labortechnik, D-7633 Seelbach, West Germany) ultrasonic processor. The Schimadzu Electric Balance (type AGE-220, Schimadzu, Kyoto, Japan).

2.2. Standard and test Solutions

Stock standard solutions:

TBN, GUF, and BHN were each dissolved in 100 mg of distilled water to provide a stock solution, with the total volume of all three solutions being 100 ml. After diluting the stock solutions of TBN, GUF, and BHN with distilled water, the final concentrations were 40 µg/ml. small amounts of TBN, GUF, and BHN were pipetted onto diamond cell ATR accessories (ZnSe) using micro pipettes for simultaneous ATR-FTIR measurement. The FTIR spectra were used to record the absorbance values at the peaks of interest for each. To create the calibration curve, we tested each location three times and averaged the results. All the distinctive peaks were plotted on calibration curves for each of the standards. The calibration curve that provided the best match was

used to determine the wavelength used to evaluate the samples. In the same way, we tested the samples and used the calibration curve to figure out how much of each substance was found in the contraband. Scanning between 4600-400 cm^{-1} allowed us to determine the absorbance amplitude of these solutions.

Preparation of calibration curve:

The absorbance or amplitude was compared to the concentration of each medication at 20 µg/mL to generate a calibration curve for each. Three separate series of 10 ml volumetric flasks were filled with stock solutions of TBN, GUF, and BHN, and then diluted to a concentration of 2-10 µg/ml with distilled water. Using a micropipette, 10 L was deposited on the diamond composite ATR crystal (ZnSe). Between 4000 and 500 cm-1 of the IR spectra were captured. Absorbance was measured and scanned, and both values were recorded. Absorbance was determined at 1151cm^{-1} for TBN (supplementary figure-2), 1332cm^{-1} for GUF (supplementary figure-4), and 919 cm^{-1} for BHN (supplementary figure-6), after the spectra were transformed to first order derivative spectra ($\Delta\lambda$=1, scaling factor=10).

Assay of Laboratory prepared mixtures:

Stock solutions of TBN, GUF, and BHN were each diluted to a specific concentration in distilled water, and then individual aliquots were added to a series of 10 ml volumetric flasks. Using a micropipette, 10 L of each solution was applied to the diamond composite ATR crystal (ZnSe). After recording the IR spectrum. TBN, GUF, and BHN concentrations were determined by using individual regression models (supplementary figure-6).

Assay of marketed formulation

Correctly transferring the cough syrup equivalent of 5 ml into a 100 ml volumetric flask, adding 6 ml of water, and sonicating the mixture for 10 minutes at room temperature. Whatman filter paper was used to strain the fluid. Finally, 1 µg/ml of TBN, 4 µg/ml of BHN, and 50 µg/ml of GuF were obtained by further diluting the stock solution. A micro pipette was used to deposit a little quantity of each standard onto the diamond cell ATR accessory (ZnSe), where the spectra could be recorded. Using FTIR spectroscopy, we were able to capture the absorbance values of the most prominent peaks in each (supplementary figure-8).

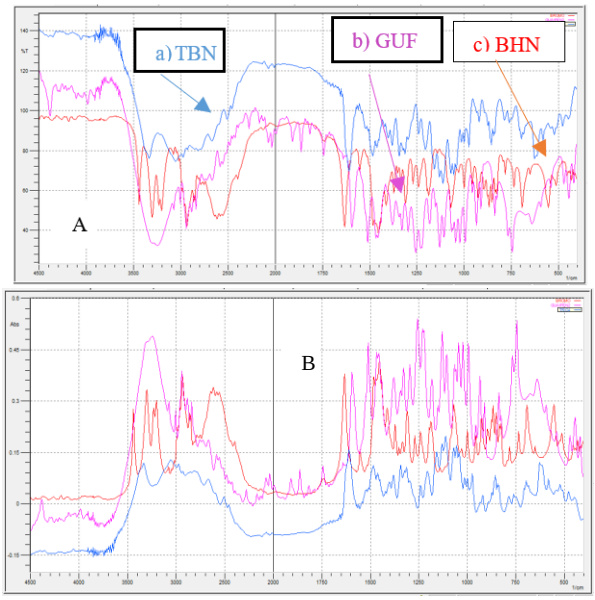

Figure-5 : Overlapped of Zero order ATR-FTIR (A) transmittance spectrum (B) ATR-FTIR absorption spectrum for (a) ***TBN,***(b)GUFand (c)BHN

3. Results and Discussion

Due to the fact that TBN, GUF, and BHN are recognized as official in IP, BP, and USP, and because some analytical methods have been reported for simultaneous estimation of compounds combination in tablet and syrup dosage form by HPTLC [14], HPLC [15-17], Spectrofluorimetric [18], and UV spectrophotometric [19], these three components were chosen for the present study's multicomponent dosage form. Attempts were attempted to estimate TBN, GUF, and BHN using ATR-FTIR, even though no quantitative approach had been created. For both qualitative and quantitative evaluations of TBN, GUF, and BHN in distilled water as genuine powder individually and in their dose form, FTIR spectroscopy is widely regarded as the best analytical approach. To make FTIR spectroscopy a more accessible quantitative liquid analytic tool, using ATR as the primary FTIR sampling technique, which, in most cases, obviates the need for sample preparation, hence saving a great deal of time during analysis. [20,21] This method gives a straightforward means of controlling several sample-handling issues and delves deeply into the analytical potential of mid-IR spectroscopy in several fields. Regardless of the quantity of analytes contained in a solution, this kind of sample preparation approach may circumvent the common difficulties associated with solvent IR absorption, which are of particular importance for aqueous solutions [22]. Additionally, the approach adopted protects fragile crystal materials by obviating the need to apply any pressure to the sample surface during handling. Calibration, validation, and analysis of unknown samples were carried out to gain better separation of all the analytes in FTIR during stress

investigations from the three active ingredient amplitude. The days of modeling before calibration. [23].The zero order spectra of TBN, GUF, and BHN showed severe overlap, as shown in (figure-5); therefore, determination of each drug was accomplished using the first derivative spectra in ATR-FT-IR by measuring a peak at 1151cm-1 for TBN where both GUF and BHN displayed zero value and by measuring a peak at 1332 cm-1 for GUF where both TBN and BHN displayed zero value (figure-6). The linear consistency of the calibration curves was shown by the excellent correlation coefficient. Standard deviations for the slope and intercept (Sb, Sa), as well as root mean square error of calibration (RMSEC) values, were calculated from the analytical data of the calibration curve in accordance with ICH [24] requirements (Table-1). The conventional addition technique was used to study the drug's recovery at three concentrations, and the pre-analyzed syrup was studied independently to determine its recovery percentage (Table 2). The high value of R2 and low values of RMSEC show that the FTIR spectra using these peaks provided accurate and precise results for quantitative measurement of TBN, GUF, and BHN. According to (Table-3), there was no statistically significant difference between the FTIR and UV data (P > 0.05). (Table-2). No statistically significant differences were seen between the overall peak concentrations and those of the individual medications in the laboratory-prepared combination for the three medicines (figure-7). Mean relative errors < 5% w/w were also found in all three sets of blind samples, adding weight to the argument that ATR-IR is a reliable approach for determining moisture content. Because a fast, reliable, and inexpensive analysis may be performed without the requirement for complex data processing techniques, the method is easily accessible and straightforward to adopt in research laboratories for regular analysis without dedicated professional staff. This exploratory work indicates the potential of ATR-IR to facilitate the development of green chemistry processes in the beauty and pharmaceutical sectors, where the usage of plant extracts carries a significant cost and maybe health risk.It was shown that the first derivative absorbances or amplitudes of the aforementioned chosen bands may be employed for the interference-free determination of the aforementioned medicine (cough syrup) (figure-8). It was determined that the percentage assay and recovery of TBN, GUF, and BHN in syrup were all adequate based on the data reported in (Table-3) The FTIR spectra of samples were optimized to determine the optimal area for predicting the concentrations of three substances using FTIR with the lowest errors and an acceptable correlation to the measured concentrations.

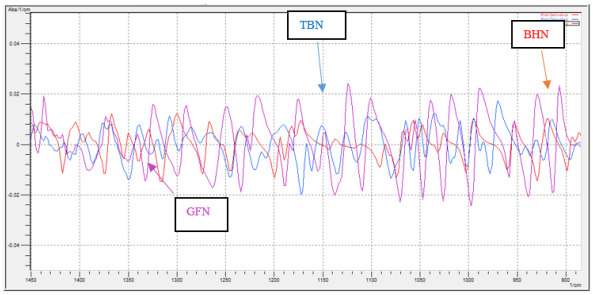

Figure-6: Zoomed spectrum area of first derivative ATR-FTIR absorption spectrum of TBN at 1151 cm^{-1}, BHN at 919 cm^{-1} and GFN at 1332 cm^{-1} .

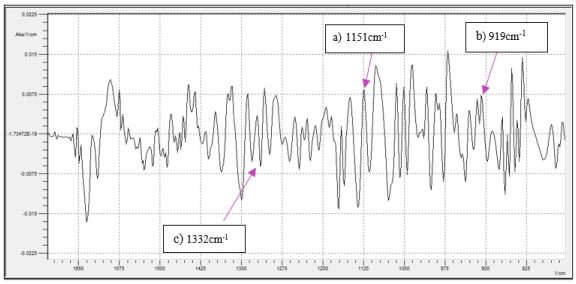

Figure-7: **Zoomed** spectrum area first derivative ATR-FTIR absorption spectrum for a) TBN, b) BHN and c) GFN mixture.

Table-1: Analytical Validation Parameters

Parameters	**TBN**	**GUF**	**BHN**
Amplitude (cm^{-1})	1151	919	1332
Beer's law range(µg/ml)	10-50	20-100	10-50
Standard regression equations (n=5)	0.1499x + 0.0023	0.1443x - 0.0286	0.1374x - 0.0298
Correlation Coefficient	0.9996	0.9998	0.9995
LOD(µg/ml)	0.0036	0.00117	0.0012
LOQ(µg/ml)	0.0011	0.00534	0. 0033
RMSEC (mg%)	0.4690	0.0063	0.0640
Inter-day % RSD	0.2650	0.1521	0.2570
Intra-day % RSD	0.2241	0.1732	0.2311
Laboratory prepared mixture	100.12±0.787	99.66±0.690	99.87±0.769
50 % Add^n ± RSD n=5	101.06± 0.1547	100.15±0.7413	100.6±0.2413
100 % Add^n ± RSD n=5	101.26±0.9504	100.80±0.5084	101.36±0.6169
150 % Add^n ± RSD n=5	99.86+0.8154	99.41+0.7621	98.41+0.3241

Table-2: Assay of marketed formulation:

	TBN	GUF	BHN
Label claim (µg/5ml)	1.25	50	4
Mean Amount found (%)	100.3±0.278	99.50±0.568	99.87±0.374
R.S.D. (%) n=5	0.3781	0.2506	0.3912

Table-3: Statistical comparison between the proposed ATR-FTIR method and the reported method for determination of **TBN, GUF,** and **BUN**, in drug substance and the reference methods:

Statistical term	UV Reference method [1]	ATR-FTIR
Mean	99.78	100.05
± S.D.	0.7041	0.4716
± S.E.	0.3140	0.2102
% R.S.D.	0.7052	0.4700
n	5	5
V	0.4955	0.2212
t (*2.23)	0.8145	
F(*5.05)	1.2304	

4. Method Validation and data analysis

Before measuring a sample, a spectrum of air (20 scans) was taken and automatically compared by the program with the sample spectrum (20-40 averaged scans). Three deposits have been measured for each sample, and three spectra have been taken from each drop. In the end, nine spectra were taken from each sample to fully capture the inter- and intra-measurement variability. Using the same settings, spectra of pure substances have also been acquired. It takes around 30 seconds to complete the full procedure, which includes cleaning the ATR crystal, gathering background data, and taking the IR spectrum of the sample.

The recovery experiments were conducted using the standard addition technique of medication at three different levels (50%, 100%, and 150%) to evaluate the efficacy of the suggested approach. Pre-analyzed syrup was spiked with known quantities of TBN, GUF, and BHN, and the percentage recoveries were determined. (Table-1).

Method accuracy (Precision) was calculated as a relative standard deviation based on intra-day repeatability and inter-day intermediate precision (RSD). Concentrations of TBN (40 µg/ml), GUF (40 µg/ml), and BHN (50 µg/ml) were measured at three separate times during the same day under the identical experimental circumstances to assess intra-day precision. This accuracy was measured by comparing the concentrations of each medication on three separate days. (Table-1).

To study the specificity of this method overlaying the spectra of the genuine solutions and the pharmaceutical dosage forms extracts demonstrates that three of the cited drugs were successfully determined by the presented methods in admixture; the three drugs were also determined in their dosage forms without any interference from the excipients (figure-8). The regression equation of the calibration was also used, and its data is presented in (Table-1).

Standard solutions of TBN, GUF, and BHN were generated in a range of concentrations from (10-50µg/mL), (20-100µg/mL), and (10-50µg/mL) correspondingly, and five concentration levels of linearity test solutions were created for the assay technique and the related substance method (figure-9). Three separate analyses were performed on each concentration. Least square linear regression was used to examine the peak area versus concentration data, and a strong correlation coefficient was used to confirm the linearity of the calibration curves. The calibration curve's analytical data, including slope and intercept standard deviations (Sb, Sa) was summarized in (Table-1).

Capacity to detect Under the parameters of the experiment, the limit of detection LOD for the analyte is determined to be 3.3 (Standard Deviation/Slope) with injections of progressively smaller amounts (but not necessarily less quantities). TBN, GUF, and BHN have detectable concentrations of 0.003 µg/ml, 0.001 µg/ml, and 0.001 µg/ml, respectively, as established by standard curve analysis (Table-1).

As stated in the experimental conditions of the technique, the limit of quantitation10 (SD/Slope) is the lowest concentration of the analyte in a sample that can be determined quantitatively, by injecting decreasing quantity of drug, with acceptable precision and accuracy. For TBN, GUF, and BHN, the LOQ (lowest concentration at which a peak can be detected) was determined to be 0.001 µg/ml, 0.005 µg/ml, and 0.003 µg/ml, respectively (Table- 1).

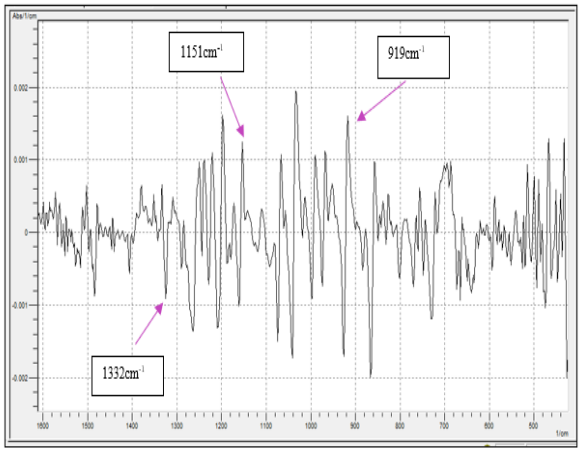

Figure-8: Zoomed spectrum area of first derivative for dosage form marketed formulation.

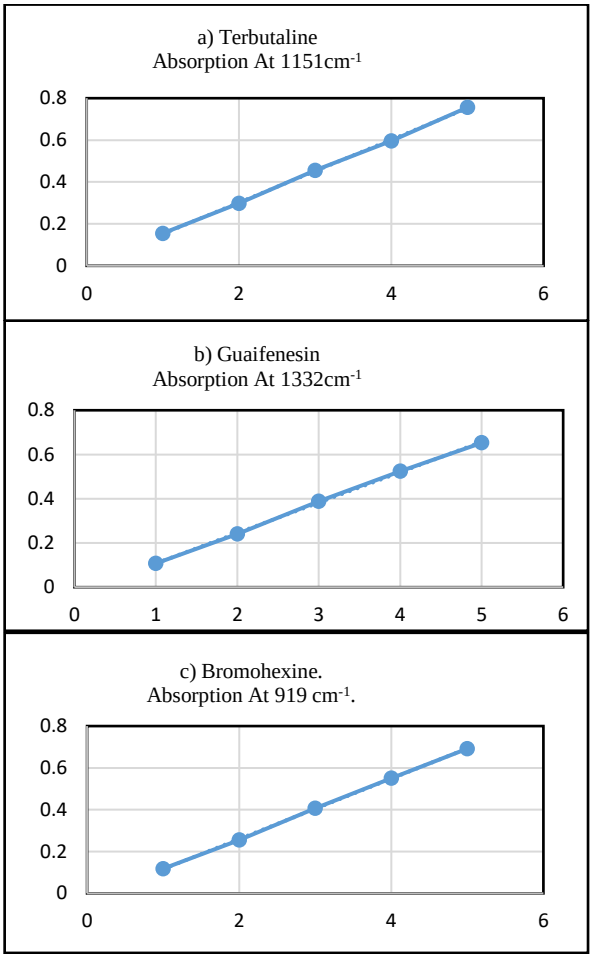

Figure-9: Calibration curve for the **determination** of (a) ***TBN,*** (b) GUFand (c)BHN using first derivative ATR-FTIR technique.

5. Conclusion

Based on the results and parameters of the above experiments, it was determined that ATR-FTIR is a more accurate method for quantifying drug mixtures than the UV-method, and that its use would be especially useful for the quality control departments of pharmaceutical companies and other organizations with claims of therapeutic efficacy. In addition, ATR's lack of solvent need removes content measurement variance due to its soluble nature. Since diluents and solvents little affect ATR-related qualitative and quantitative analyses, this research finds that ATR-FTIR quantification is more accurate than UV-Vis technique.

6. Conflicts of interest

There are no conflicts to declare.

7. Acknowledgement

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