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RESEARCH ARTICLE

Analysis of Raspberry Ketone in Nutraceutical Formulation Using Fourier Transform Infrared Spectrophotometric Method

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Abstract:

Aims:

A Fourier Transform Infrared (FT-IR) spectrometric method was developed for the rapid, direct measurement of Raspberry Ketone (RK) and Caffeine (CAF) in a nutraceutical formulation.

Methods:

Conventional KBr-spectra and KBr+0.5 mg Microcrystalline Cellulose (MCC)-spectra were used as the basis for a better determination of active substances in the nutraceutical formulation. A calibration model was developed using caffeine and raspberry ketone standards of varying concentrations in the mid-infrared region (4000-400 cm^{-1}). The Beer-Lambert law was used in data processing.

Results:

The results indicate that FT-IR spectrometry is applicable to the analytical quantification of RK and CAF in the nutraceutical formulation.

Conclusion:

The method proposed is simple, precise and not time-consuming compared to the chromatographic methods that are cited in the literature. Quantification is performed in about 10-15 minutes, including sample preparation and spectral acquisition.

Keywords: Microcrystalline cellulose, Data processing, Ketone standards, Spectrometric method, Nutraceutical formulation, Analytical quantification.

1. INTRODUCTION

Infrared spectroscopy is a standard analytical technique used to analyze any type of substances, thereby generating images of all atomic vibrations in the compound. Therefore, it is also referred to as vibrational spectroscopy. A classical infrared spectrum is obtained by passing an infrared beam through a sample and then determining the fraction of incident radiation absorbed by the sample at a given wavelength.

Infrared spectrometry allows the identification of pharmaceutical compounds as well as their quantitative analysis and the use of the FTIR technique ensures the continuous monitoring of the baseline and the simultaneous analysis of several different components in the same sample [1 - 8]. FTIR is an efficient technique for pharmaceutical analysis because it is easy to use, sensitive, and fast.

Raspberry Ketone (I, RK) (also known as 4-(4-hydroxyphenyl) butan-2-one) is a compound extracted from red raspberries that is usually used as a scenting and flavoring agent in foods and cosmetics [9].

1.1. Chemical Structures

Numerous researches have been carried out to determine the biological effects of the raspberry components, thus obtaining numerous results. For example, the ellagic acid is

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reported to inhibit tumor inductions in the liver, lungs and esophagus only in tumour cell lines and animal models [10]. It is presumed that RK might influence the lipid metabolism in ways similar to capsaicin and synephrine. The effects of RK on obesity and lipid metabolism were examined by Morimoto *et al.* [11].

RK is found naturally in many foods, most notably raspberries, but also in Rheum officinale [12].

Dietary intake is estimated to be about 0.42mg/kg bodyweight [13], consumed through processed foods where raspberry ketone is added as a flavoring agent.

Raspberry ketone quantitative determination has been performed using using polarographic technique polarography [14], spectrometry [15] and liquid chromatography - mass spectrometry [16 - 18]. The-HPLC separation of raspberry ketone and caffeine on phenyl columns (PhE and PPF) has also been described [16].

Caffeine (II, CAF) (3, 7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione or 1,3,7- trimethylxanthine) is a central nervous system and metabolic stimulant, which is used both recreationally and medically to reduce physical fatigue and to restore alertness when drowsiness occurs [19 - 21]. It produces increased wakefulness, faster and clearer flow of thought, increased focus, and better general body coordination. Caffeine's most notable pharmacological effect is as a central nervous system stimulant, increasing alertness and producing agitation. It also acts as a urinary stimulant.

Caffeine is used in some headache and migraine medications, in certain dietary supplements used for weight loss and in many popular energy drinks [22].

Caffeine quantitative determination has been performed using different techniques ranging from liquid chromatography to spectrometry [23 - 28].

The main objective of this work was to develop a new procedure for the fast and accurate simultaneously determination of RK and CAF in commercial nutraceutical formulations containing 100 mg RK and 50 mg CAF per tablet, by using the Beer-Lambert law thus reducing the steps required for sample pre-treatment and providing direct FTIR measurement.

2. EXPERIMENTAL

2.1. Apparatus

Data acquisition was performed using a Frontier Two System FT-IR spectrometer equipped with Spectrum for Windows v.5.01 (Perkin Elmer Co., Beaconsfield, Bucks, UK). The commercial softwares used were QUANT+ expert v/4.51 and Spectrum Beers's law v.2.01 (Perkin Elmer Co.,UK) to generate data for the principal component analysis. All the FT-IR spectra were scanned between 4000 and 400 cm^{-1}, by averaging 32 scans for each spectrum with a resolution of 4 cm^{-1} (data point resolution/interval 1 cm^{-1}) and were recorded with a DTGS detector.

2.2. Reagents and Materials

The standards of RK, CAF and Microcrystalline Cellulose (MCC) were supplied by Sigma Aldrich. For fused KBr disk preparation a potassium bromide IR spectral grade was used (Sigma Aldrich, Steinhein, Germany). The nutraceutical formulation, Ultimate containing 100mg RK/tablet and respectively 50mg CAF/tablet, manufactured by WELLNX – Willington Delaware, USA, was purchased from the local market.

The principal excipient used in the tablet formulation is Microcrystalline Cellulose (MCC).

2.3. Recommended Procedures

The standards were prepared by compressing 2.0 mg of substance with spectral grade KBr (up to 200mg), while the background was spectral grade KBr. Each spectrum was collected three times for the same cup after rotation with 120. The mean of the spectra was then used in the following analysis steps.

For the calibration curve of RK, we used samples prepared by compressing a standard substance RK in spectral grade KBr, while the background was 0.5 mg MCC in KBr (calibration was made using four levels: 0.1, 0.2, 0.3 and 0.4 mg respectively). For the calibration curve of CAF, spectra were recorded from samples prepared by compressing a standard substance CAF in spectral grade KBr, while the background was 0.5 mg MCC in KBr (calibration was made using four points 0.05, 0.1, 0.15 and 0.2 mg respectively).

For the drug determination, the spectra were performed in two different ways:

- Conventional fused KBr disk spectra were recorded from the samples prepared by compressing 2.0mg of drug sample with spectral grade KBr, while the background was spectral grade KBr [Drug -KBr].
- Conventional fused KBr disk spectra were recorded from samples prepared by compressing 2.0 mg of drug sample with spectral grade KBr, while the background was 0.5mg MCC in KBr [Drug-MCC].

Experimental parameters, such as calibration methods, were compared and recommendations on the best options for RK and CAF analysis were made.

3. RESULTS AND DISCUSSION

Fig. (**1**) presents the mean spectra for RK and CAF standards using the KBr disk method while the spectra of the nutraceutical formulation are presented in Fig. (**2**).

We studied the possibility of using the Beer-Lambert law for the quantitative determination of RK and CAF in nutraceutical tablet formulation at 1516 cm^{-1} for RK, corresponding to p-OH stretching, and at 746 cm^{-1} for CAF, corresponding to amino group. Figs. (**3 and 4**) present the calibration graphs for the active principle RK and CAF respectively, taking into account the peak area calculated.
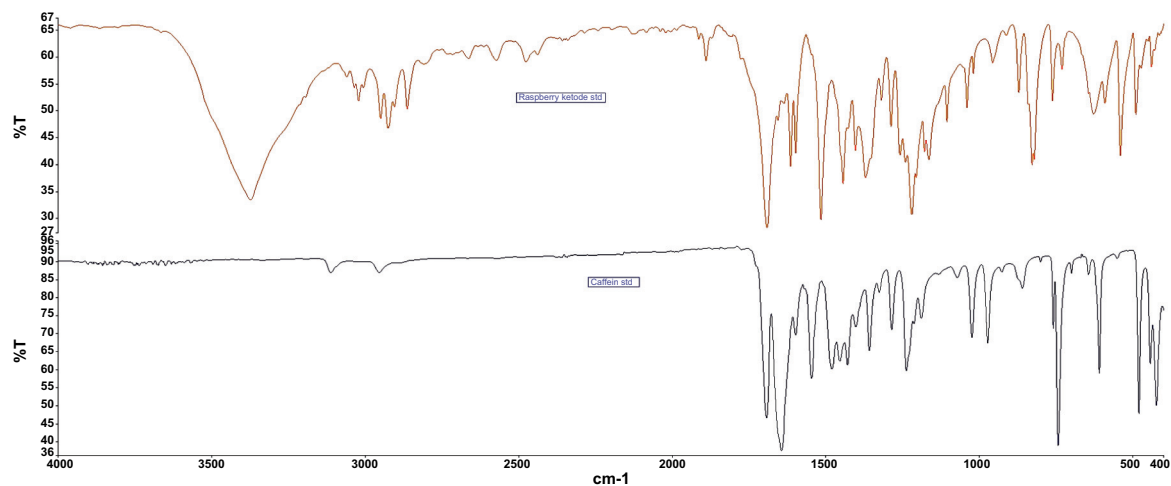
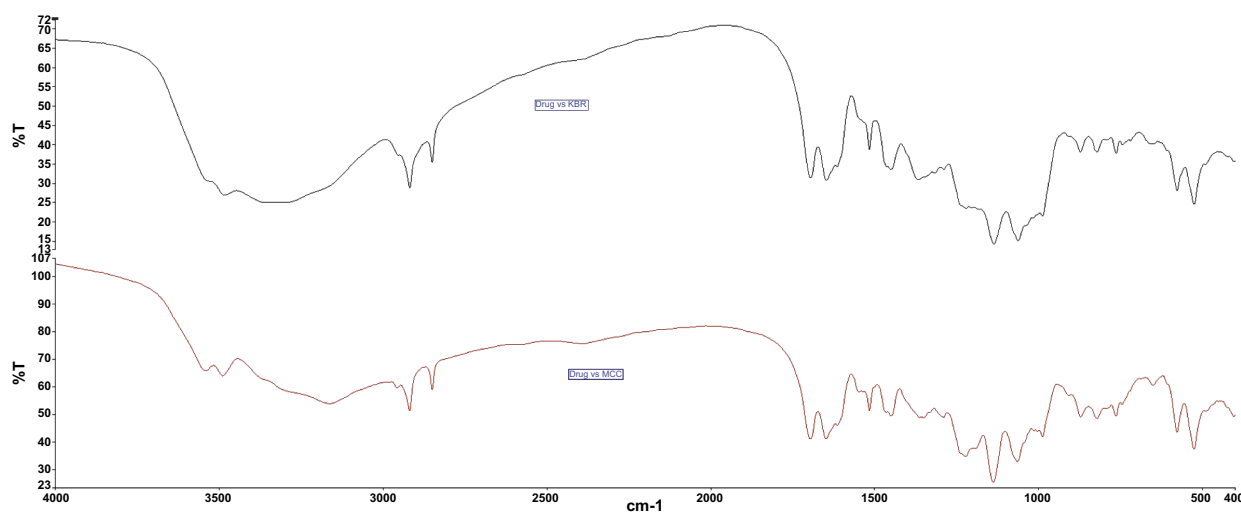**Fig. (1).** FT-IR spectra of RK and CAF standards in KBr-disk.

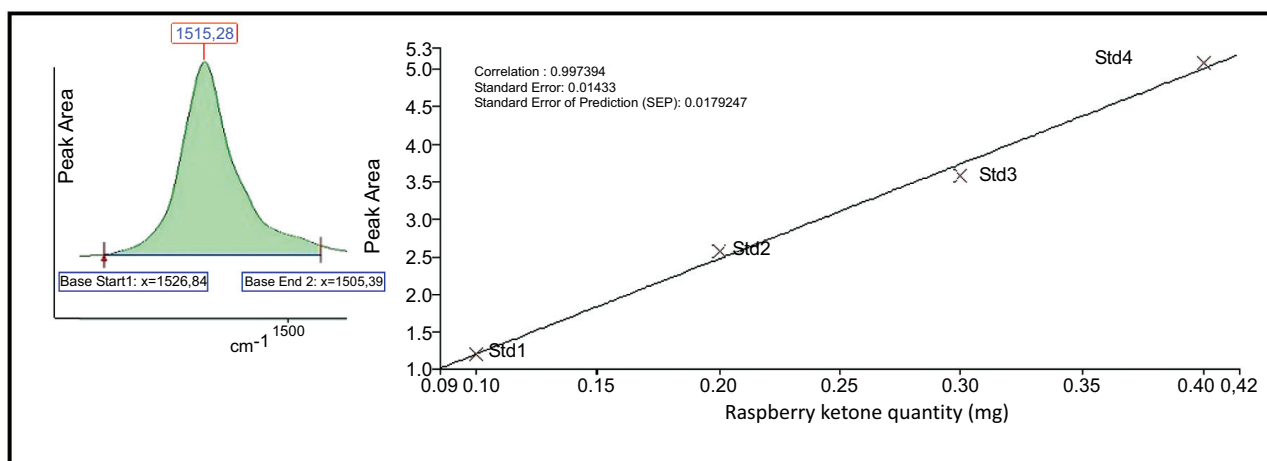**Fig. (2).** FT-IR spectra of drug formulations.

Fig. (3). Beer-Lambert law calibration curve for RK determination in pharmaceutical preparations.
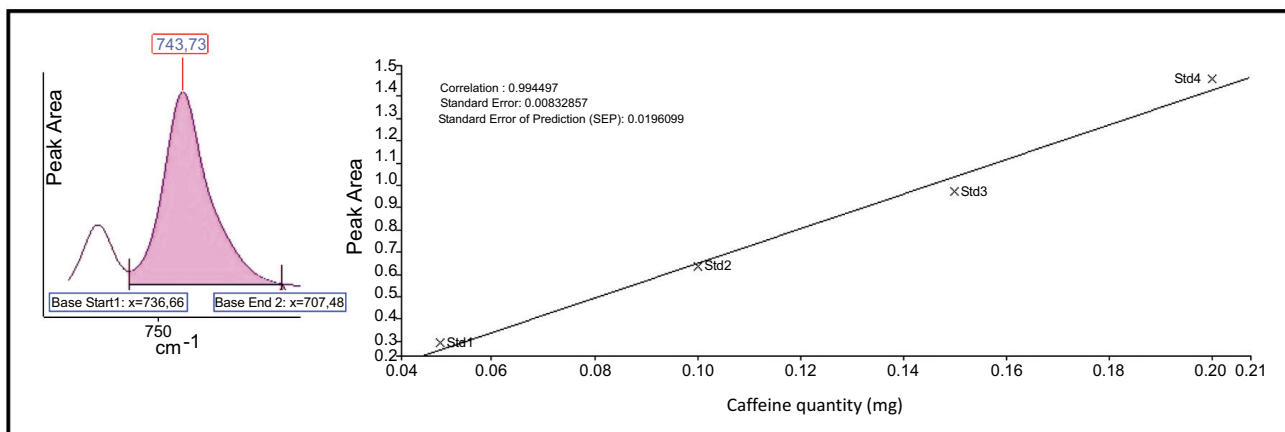
Fig. (4). Beer-Lambert law calibration curve for CAF determination in pharmaceutical preparations.

The measurements, carried out under the previously mentioned conditions, providing a typical calibration line which corresponds to:

A= - 0.0367 + 12.6278 C (mgRK) (R^2 = 09974)

A= - 0.1251 + 7.7848 C (mgCAF) (R^2 = 09945)

As can be seen in Table **1**, the results are very similar, and we suggest the use of the Beer-Lambert law method, because of the smaller value of RSD (< 3.0%).

For the validation of the method the principal parameters of the procedure were studied.

The linearity was studied for both analytes, and as presented in Figs. (**3** and **4**) good correlation coefficients were obtained (R^2>0.995).

The sensitivity of the method was established by determining Limits of Detection (LOD) and Limits of Quantification (LOQ). Both parameters were determined according to the ICH guidelines [29]. The low LOD and LOQ values presented in Table **2** show the good confirm the good sensitivity of the method.

Table 1. Results for the Supplement formulation determination in tablets using FT-IR chemometric approaches.

	SUPPLEMENT FACTS			
–	RK (mg/tablet)		CAF (mg/tablet)	
	Theoretical	Found	Theoretical	Found
Content	100.0	99.5300	50.0	49.6967
RSD (%) (n=6)	-	2.51	-	2.67

Table 2. Results for the validation procedure in analytical determination of RK and CAF using FT-IR chemometric approaches.

	SUPPLEMENT FACTS			
–	RK (mg/tablet)		CAF (mg/tablet)	
	Theoretical	Found	Theoretical	Found
Content	100.0	99.5300	50.0	49.6967
RSD (%) (n=6)	-	2.51	-	2.67
LOD	-	6.55×10^{-5}	-	1.13×10^{-4}
LOQ	-	1.05×10^{-5}	-	3.34×10^{-4}
Inter-day measurements				
RSD (%) inter-day	-	2.17	-	2.25
RE (%)	-	0.81	-	0.99

The precision of the method was studied in two days inter-day measurements over two days. The values of RSD and RE, presented in Table **2**, indicate a good precision of the method. proposed by us.

In Table **2** all the results obtained after the validation of the FTIR method are presented.

CONCLUSION

The results indicate that FT-IR spectrometry can be employed for the analytical quantification of RK and CAF in a nutraceutical formulation using commercial software. The method proposed is simple, precise and not time-consuming compared to the chromatographic methods cited in the literature. Quantification is performed in about 10-15 minutes, including sample preparation and spectral acquisition.

ETHICS APPROVAL AND CONSENT TO PARTI-CIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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