Analytical, Nutritional and Clinical Methods

Rapid determination of caffeine content in soft drinks using FTIR–ATR spectroscopy

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Abstract

A simple and rapid Fourier Transform infrared (FTIR) spectroscopic method was developed to determine caffeine content in soft drinks without the use of organic solvents. FTIR spectrum of carbonated beverages were characterized and the region between 2800 and 3000 cm^{-1} was used for quantitative estimation using partial least square (PLS) and principal component regression (PCR). FTIR spectroscopy with chemometrics, using the PLS-1st derivative spectra could predict the caffeine content accurately upto an R^2 value greater than 0.97 and a standard error of prediction (SEP) of less than 2.43 with 4–6 factors in the prediction model. The developed model was applied to predict caffeine content in four commercial carbonated beverages in approximately 5 min. The developed procedure was further validated by recovery studies by comparing with UV spectroscopic method. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: FTIR spectroscopy; Caffeine; Carbonated beverages; Chemometrics

1. Introduction

Methylxanthine, an alkaloid, consists of compounds such as caffeine, theophylline, and theobromine, and are normally found in cola nuts, coffee, tea, cacao beans, mate and other plants. Chemically caffeine is a 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6,-dione or 1,3,7-trimethylxanthine. Global consumption of caffeine has been estimated to be 120,000 tonnes per annum, approximately equivalent to one caffeine containing beverage per day by each of the planet's 5 billion plus human inhabitants (Hopes, 1997). Hence, caffeine is almost certainly the most widely consumed psychoactive substance in the world. Caffeine content in soft drinks varies by brand, but the US Food and drug Administration (FDA) limits the maximum amount in carbonated beverages to 6 mg/oz. Therefore, caffeine content allowed in a 355 ml (12 oz) can of soft drinks is 72 mg. Hence soft drinks may contain caffeine in the range between 30 and 72 mg per 355 ml or 8.45–20.28 mg/100 ml (National Soft Drink Association, 1999).

The above reported facts have made it essential for manufacturers to monitor and assess the concentration of caffeine in their respective products. Quantitative estimation of caffeine has been attempted using several methods. These include HPLC (Deandrade, Pinheiro, Lopes, Martins, Amorim, & Brandao, 1995; Mahesan & Lai, 2001; Naik & Nagalakshmi, 1997), UV spectroscopy (Lau, Luk, Cheng, & Chiu, 1992), TLC-MS (Prosek, Golc-Wondra, Vovk, & Andrensek, 2000) and gas chromatography (Conte & Barry, 1993; Yang, Orton, & Pawliszyn, 1997). Capillary electrophoresis has also been used for quantitative determination of caffeine in beverages (Hurst & Martin, 1993). All of the above reported methods are accurate but somewhat expensive and time consuming and requires sample preparation prior to analysis. Hence there is a need for a low-cost, simple, and rapid method of caffeine determination.

Flow injection Fourier transformed infrared method (Daghbouche, Garrigues, Vidal, & Guardia, 1997) have been used to determine the amount of caffeine in soft drinks. Here the sample was passed through a C_{18} SPE cartridge and extracted in chloroform prior to FTIR analysis. The present study proposes to develop a rapid and simple technique to estimate the amount of caffeine

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in commercial products without any sample treatment. The main objective of this study was to investigate the potential of FTIR spectroscopy to determine caffeine content in soft drinks and to validate the model by a conventional method and recovery study.

2. Materials and methods

2.1. Samples

Caffeine free soft drink samples of different brands such as Coca Cola classic[TM] and Sprite[TM] (The Coca-cola Company, Pittston, PA, USA), Pepsi[TM], and Mountain Dew[TM] (PepsiCo, Inc., Somers, NY, USA) as well as normal caffeine containing samples of the available brands were obtained from local market. Pure anhydrous caffeine (Sigma Chemical Co., MO, USA) was used for calibration studies.

Caffeine free soft drink products mentioned above were used to prepare samples with different concentration of caffeine expressed as a percentage by weight of soft drinks. Admixture sample set included 60 samples for each selected soft drink in the concentration range between 0 and 30 mg/100 ml with increment in steps of 0.5 mg/100 ml of caffeine. The ranges were chosen to evaluate the adequacy of the method for caffeine quantification studies in soft drinks. Forty-six of these samples were used for calibration, the remaining 15 were used for validation. Samples were mixed well and kept at room temperature to equilibrate before FTIR measurements.

2.2. FTIR analysis

A Bio-Rad FTS 6000 (Cambridge, MA) spectrometer equipped with a deuterated triglycine sulphate detector was used for FTIR analysis. The sampling station was equipped with an overhead ATR accessory (Horizontal Attenuated Total Reflectance Accessory with multiple reflections i.e. 10) comprising of transfer optics within the chamber through which infrared radiation is directed to a detachable ATR zinc selenide crystal mounted in a shallow trough for sample containment. Distilled water was used to obtain the background spectra, and 256 coadded scans were taken at a resolution of 32 cm^{-1}. Single beam spectra (4000–400 cm^{-1}) of the samples were obtained, and corrected against the background spectrum of water, to present the spectra in absorbance units. The ATR crystal was carefully cleaned with water between measurements and dried using nitrogen gas after each experiment to ensure the best possible sample spectra. Spectra were collected in duplicate and used for multivariate analysis. Commercial samples of carbonated soft drinks were degassed and then used for FTIR analysis. Each experiment was repeated three times.

2.3. Chemometrics: multivariate analysis

Multivariate analysis was used for quantitative and qualitative analysis. Partial least squares (PLS) and principle component regression (PCR) algorithms, proven to be effective in many quantitative applications were used in the present study. The Grams 32 (Galactic Industries Corporation, Salem, NH) software was used for PLS (Haaland & Thomas, 1988) and PCR (Martens & Naes, 1988) analysis. Calibration models with original and 1st derivative transformed spectra were developed and the optimum number of calibration factors was selected based on the predicted residual error sum of squares (PRESS), which should be minimized, along with the R^2 from regression which should be maximum. The predictability of the models was tested by computing the standard error of calibration (SEC) for the calibration data set and standard error of prediction (SEP) for the validation data set.

2.4. Preparation of calibration and validation models

Caffeine free soft drinks samples were divided into two parts. One part was kept without any caffeine and the other (100 ml) used to dissolve 50 mg of anhydrous caffeine powder. The two solutions were then blended in the correct proportion to give 61 samples of varying caffeine concentration in the range between 0 and 30 mg/100 ml, in steps of 0.5 mg. Total volume of each sample prepared was 5 ml.

When preparing the samples for chemometric analysis the rule of thumb (the number of PLS factors should constitute one-sixth the number of independent specimens in the calibration set) stated by Kemsley (1998) was adopted. Approximately 45 samples (different concentrations) were used to avoid over-fitting of the calibration model and rest were used for validation.

2.5. Estimation of caffeine by UV spectrometric method

Degassed soft drink samples (10 ml) were taken in separating funnels and an equal amount of chloroform was added to each sample. The separating funnel was shaken vigorously for 5 min and the solutions were allowed to separate for 10 min at room temperature. One milliter of the chloroform layer (lower) collected from separating funnel, was diluted with 4 ml of pure chloroform and was used for analysis. Absorbance of these solutions was measured at 277 nm (i.e. at predetermined λ max) against pure chloroform as blank using the Perkin Elmer lambda 40 UV–Vis spectrometer (Perkin Elmer, Uberlingen, Germany). A standard curve was prepared for caffeine concentrations in the range between 0 and 0.2 mg/ml.

2.6. Recovery studies

The validity and accuracy of the newly developed methods was further explored through recovery studies.

Recovery study involves, artificially spiking the samples analyzed with the target analyte (e.g. caffeine) and predicting its content using the method developed. Here 10, 20, and 30 mg/100 ml of pure caffeine was artificially introduced into caffeine free carbonated drinks such as Coca Cola (classic), Sprite, Pepsi and Mountain dew and the caffeine content was estimated by FTIR and UV spectrometric methods as described in Sections 2.2 and 2.5. The results were expressed as % recovery.

3. Results and discussion

3.1. Characterization of FTIR spectrum of soft drinks

Fig. 1 presents the ATR spectra of different caffeine free soft drinks with added caffeine. Coca Cola with spiked caffeine shows absorbance bands at 818, 866, 907, 1059, 1253, 1341, 1426, 1507, 1649, 1714, 2829, 2882, 3218, 3564 and 3741 cm^{-1}. ATR spectra of all the
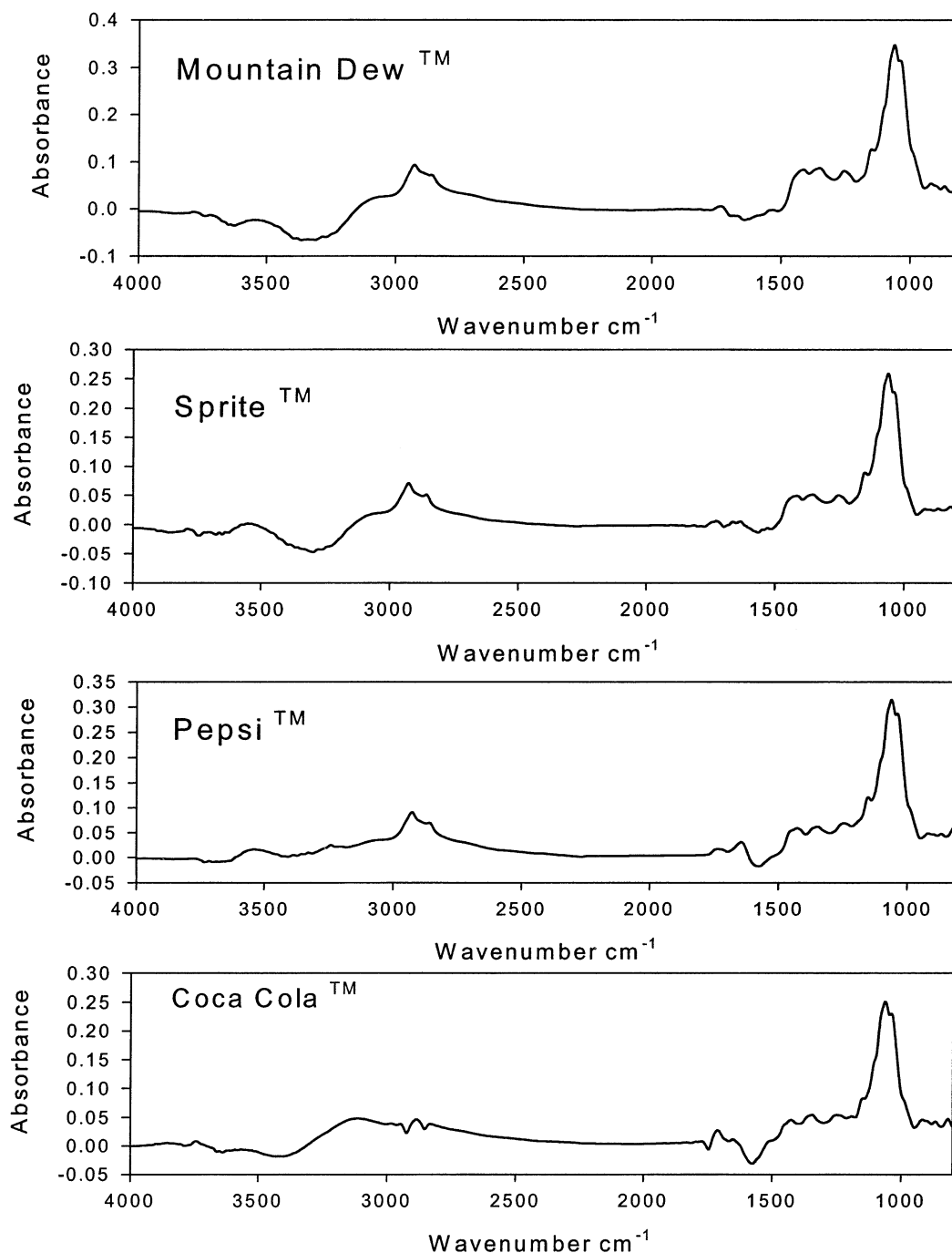

Fig. 1. FTIR spectra of different soft drinks.

other soft drinks represent peaks similar to that of Coca Cola. Most soft drinks contain sugars (high fructose corn syrup and or sucrose), phosphoric acid, citric acid, and carbonated water as common ingredients, whereas caramel color is present only in Coca Cola and Pepsi and concentrated orange juice in Mountain Dew. Similarity of the major ingredients gives similar peak positions in the ATR spectra of the soft drinks studied.

The structure of caffeine comprises of 10 C–H bonds (including those from three CH_3 groups consisting of nine C–H bonds), two C=O bond (carbonyl group on cyclic structure), one C=C bond (only unsaturated C–C bond in cyclic structure), one C=N bond, 10 C–N bonds and one C–C bond. Peak at 2829 cm^{-1} may be due to C–H bond of aldehydic sugars such as glucose whereas peak at 2882 cm^{-1} found to be significant in caffeine estimation studies, may be due to the stretching vibration of bonds from multiple constituents from the soft drinks. These vibrations include C–H stretching of hydrocarbons such as carbohydrates and citric acid, O–H stretching of carboxylic acids such as citric acid and most importantly asymmetric stretching vibration of C–H bonds of methyl (–CH_3) groups. Caffeine molecule has the characteristic three methyl group cyclic structure and hence this peak may play an important role in the estimation of caffeine.

3.2. Identification of significant region of spectra for analysis

Table 1 shows the PLS analysis of different region of the soft drinks spectra. When the full spectra between 800 and 1200 cm^{-1} were used, R^2 values greater than 0.97 was obtained for the soft drinks (Coca Cola, Pepsi, Sprite and Mountain Dew) considered. This region includes a major peak at 1061 cm^{-1}. The region between 1200 and 1600 cm^{-1} (with minor peaks at 1253, 1341, 1426, and 1507 cm^{-1}) gave a lower R^2 value for all the soft drinks than the representation using the 800–1200 cm^{-1} region of the spectra. The next region between 1600 and 2000 cm^{-1} gave a lower R^2 value, with a minimum around 0.93.

Table 1
R^2-values for PLS models using different spectral region of artificially caffeinated soft drinks (originally caffeine free)

Spectral region (cm^{-1})	R^2-value			
	Coca Cola	Pepsi	Sprite	Mountain Dew
800–1200	0.9829	0.9745	0.9749	0.9680
1200–1600	0.9713	0.9739	0.9702	0.9664
1600–2000	0.9774	0.9343	0.9787	0.9433
2800–3000	0.9974	0.9751	0.9911	0.9739
3000–3600	0.9813	0.9318	0.9673	0.9664
2800–3600	0.9709	0.8412	0.9227	0.9513
800–1200 and 2800–3000	0.9881	0.9319	0.9799	0.9618

When the spectral regions between 2800 and 3000 cm^{-1} was used for analysis, maximum R^2-values obtained were greater than 0.99, 0.98, 0.99, and 0.97 for Coca Cola, Pepsi, Sprite and Mountain Dew models respectively in this region. Table 1 presents analysis of the individual regions of the spectra for prediction model development. The region between 2800 and 3000 cm^{-1} contains two peaks of 2829 and 2882 cm^{-1}, of which the later one can be correlated with the asymmetric stretching of C–H bonds of methyl (–CH_3) group in the caffeine molecule. Hence this region was used to develop calibration and validation model for quantitative analysis of caffeine in soft drinks.

3.3. Calibration model for quantitative analysis of caffeine

The PLS and PCR model performance indicators with their original and first derivative spectra as input are shown in Table 2. R^2-values for Coca Cola analysis approximates 0.99 for the calibration as well as validation data sets and the SEC and SEP estimates were lower than 1.78. The PLS with 1st derivative gave a slightly better result with SEC and SEP values of 1.22 and 1.32, respectively. The factor of analysis for the models obtained using the spectra in the 2800–3000 cm^{-1} regions was four, indicating the consistency of prediction.

In the prediction models for Pepsi, Sprite, and Mountain Dew the PLS and PLS-1st derivative models were better than other methods with R^2-values greater than 0.97 for the calibration as well as validation data sets and with SEC and SEP estimates of less than 2.82 and 2.45, respectively. Since the PLS-1st derivative was consistently a better predictor, this technique was used to further validate and test the model. The minimum "detection limit" for this FTIR caffeine estimation method was found to be as low as 0.5 mg/100 ml.

3.4. Analysis of commercial samples of carbonated beverages products

Caffeine free soft drinks such as Coca Cola, Pepsi, Sprite and Mountain Dew analyzed by FTIR spectroscopy and chemometrics was used to develop calibration models from the PLS-1st derivative method. Results obtained from FTIR spectroscopy were compared with that of the conventional UV spectroscopic method (Table 3) which requires the analyte, i.e. caffeine, to be extracted in chloroform and then used in analysis. The results obtained by FTIR method were found to be slightly higher compared to that of UV spectroscopic method. The slightly higher caffeine content values were obtained with FTIR method compared to UV method, may be due to the presence of interfering compounds. The sample required for FTIR analysis does not require any pretreatment and can be used as such.

Table 2
Chemometric analysis of soft drinks using the 2800–3000 cm^{-1} spectral region

Soft drinks	Statistical methods	Factors of analysis	Calibration model		Validation model	
			R^2	SEC	R^2	SEP
Coca Cola	PLS	4	0.989	1.672	0.992	1.490
	PLS-1st Derivative	4	0.994	1.225	0.992	1.321
	PCR	4	0.988	1.783	0.992	1.583
	PCR-1st Derivative	4	0.992	1.464	0.989	1.523
Pepsi	PLS	6	0.975	2.616	0.971	2.436
	PLS-1st Derivative	6	0.971	2.819	0.970	2.454
	PCR	7	0.973	2.764	0.968	2.527
	PCR-1st Derivative	7	0.964	3.204	0.966	2.655
Sprite	PLS	6	0.991	1.566	0.993	1.221
	PLS-1st Derivative	6	0.992	1.495	0.994	1.278
	PCR	6	0.991	1.590	0.993	1.232
	PCR-1st Derivative	6	0.989	1.734	0.990	1.489
Mountain Dew	PLS	6	0.974	2.684	0.984	1.919
	PLS-1st Derivative	6	0.983	2.164	0.986	1.761
	PCR	6	0.972	2.803	0.984	1.939
	PCR-1st Derivative	6	0.980	2.370	0.985	1.816

Table 3
Comparison of FTIR prediction of caffeine content spectroscopy prediction with the standard method

Products (caffeine free)	Caffeine content by UV spectroscopic method (mg%)		Caffeine content by FTIR method (mg%)	
	Mean	±SD	Mean	±SD
Coca Cola	0.166	0.06	1.014	0.34
Sprite	0.108	0.04	0.855	0.28
Pepsi	0.129	0.07	1.150	0.54
Mountain Dew	0.143	0.06	1.189	0.19

Table 4
Analysis of caffeine containing soft drink samples

Soft drink samples	Caffeine content (mg/100 ml)				
	UV method		FTIR method		Reported values[a]
	Mean	±S.D.	Mean	±S.D.	
Coca Cola Classic	9.749	0.16	10.055	0.47	9.577
Pepsi	10.553	0.23	10.790	0.46	10.704
Mountain Dew	15.867	0.30	16.150	0.47	15.493

[a] National Soft Drink Association (1999).

Research of caffeine estimates in commercial samples of Coca Cola Classic, Pepsi, and Mountain Dew (caffeine containing) using developed models are presented in Table 4. The caffeine content estimated by FTIR method was found to be slightly greater than that of obtained by standard UV method.

3.5. Recovery studies

The FTIR method was further validated through recovery studies by determining the caffeine content in artificial spiked (at different concentration) commercial caffeine free soft drinks. FTIR prediction in the recovery study was found to be successful and comparable to the results obtained by the UV spectroscopic method (Table 5). The UV spectroscopic method shows caffeine recovery in the range between 100.2 and 105.8%, whereas, recovery study using FTIR data with the PLS models was in the range between 90.1 and 106.5%. The caffeine recovery by FTIR method was slightly lower than that of the UV method showing a comparatively

Table 5
Results from the caffeine recovery study using the UV and FTIR spectroscopy

Commercial caffeine free soft drinks	Added caffeine (mg%)	% Caffeine recovery by UV spectroscopy method	% Caffeine recovery by FTIR method
Coca Cola	0	–	–
	10	104.0	93.6
	20	103.4	98.9
	30	100.2	97.0
Sprite	0	–	–
	10	103.9	99.3
	20	101.7	96.2
	30	101.0	100.0
Pepsi	0	–	–
	10	104.5	103.4
	20	103.0	106.5
	30	100.6	93.91
Mountain Dew	0	–	–
	10	105.8	90.1
	20	104.1	93.2
	30	102.6	96.7

wider range of variation. However, the simplicity of sample presentation and the absence of wet chemicals makes this an attractive choice.

4. Conclusions

A rapid and simple FTIR procedure was developed to directly determine the amount of caffeine content in soft drinks. PLS-1st derivative calibration models were developed using the spectra in the region between 2800 and 3000 cm^{-1}. The model was tested on artificially spiked caffeine free carbonated beverages such as Coca Cola, Pepsi, Sprite, and Mountain Dew as well as caffeinated beverages. The FTIR procedure was validated by comparing the prediction with the conventional UV spectroscopy (caffeine was extracted in chloroform and then analyzed by UV method) and also through recovery study. The FTIR method developed is not expensive and analysis could be done in less than 5 min. The technique could be used to assess the integrity of caffeine free beverages and can be extended to detect the amount of caffeine in other commercial products.

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