Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company’s public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Infrared Spectroscopic Technologies for the Quality Control of Herbal Medicines

Christian Huck

Institute of Analytical Chemistry and Radiochemistry, CCB—Center for Chemistry and Biomedicine, Leopold-Franzens University, Innsbruck, Austria

22.1 INTRODUCTION

Herbal medicine is the oldest and most widely used form of medical treatment in the world, and enjoys increasing popularity due to its health-promoting properties [1]. Medicinal and herbal plant properties are related to individual ingredients usually found in the parts-per-million (ppm) and/or parts-per-billion (ppb) range [1]. During the recent decade, the pharmaceutical industry has initiated sophisticated plant screening programs, applying biochemical high-throughput techniques to find new drugs with distinct properties (e.g., anticancer, antibacterial, and/or antiviral properties) [2]. Traditionally, separation techniques including thin-layer chromatography (TLC), liquid chromatography (LC), gas chromatography (GC), and capillary electrophoresis (CE) hyphenated to mass spectrometry (MS) were employed for the identification, quantification, and structural elucidation of selected compounds being present or deriving from different plant matrices [1,2]. These analytical techniques have been found useful in...
phytochemical and physiological studies, enabling recording a fingerprint and/or identifying single active compounds [3].

Spectroscopic analytical techniques using the near-infrared (NIR) wavelength region of the electromagnetic spectrum have been used in the food industry to monitor and evaluate on one side the composition and on the other side the quality of foods [4]. Even though the NIR region was the first known nonvisible part of the electromagnetic spectrum and was discovered in 1800 by Herschel, it was not until the 1950s that the first applications of NIR spectroscopy for analytical chemistry were developed. Since the 1960s, there has been a steady increase in the use of NIR spectroscopy, with the most dramatic growth in the last 25 years.

NIR spectroscopy is characterized by low molar absorptivity and scattering. In the beginning, the NIR region was regarded as having little potential for analytical work. In recent times, it has become one of the most promising techniques for molecular spectroscopy. Affordable and powerful computers have further supported the implementation of applications in several fields, including medical, textile, polymer, and pharmaceutical applications [5].

Vibrational spectroscopic imaging has become an essential tool for tissue analyses in life science, and can support NIR spectroscopic studies in a synergistic manner, because it allows depiction of the spatial distribution of potent ingredients. It is a modern analytical technique enabling the detection and characterization of molecular components of plant tissue samples down to a resolution of approximately 1.2 μm, applying NIR and mid-infrared (MIR) spectroscopy [6]. It is based on the absorption of Infrared (IR) radiation by vibrational transitions in covalent bonds, and enables global analysis of samples, with resolution close to the cellular level.

An advantage of vibrational spectroscopic imaging is that it can acquire local molecular expression profiles while maintaining the topographic integrity of the tissue by avoiding time-consuming extraction, purification, and/or separation steps, respectively. With this nondestructive analytical method, it is possible to obtain, on one hand, qualitative and quantitative information on heterogeneous samples, and on the other hand, unique chemimorphological information about the tissue status on the other hand. This characteristic of vibrational spectroscopic imaging represents an extremely important benefit for further interpretation of the present current tissue status.

This contribution highlights the fundamental principles of NIR and imaging spectroscopy, its applicability, regulatory issues, advantages, synergistic combination, and limitations in herbal medicine research.

### 22.2 TECHNICAL PRINCIPLES

#### 22.2.1 NIR Spectroscopy

IR radiation is the region of the electromagnetic spectrum between the visible (VIS) and the microwave wavelength [7]. In NIR spectroscopy, excitation of molecules is accomplished in a wavelength range between 750 and 2500 nm (Figure 22.1), corresponding to a wave-number range between 4.000 and 13.000/cm [8]. C–H, C–O, C=O, N–H, and O–H functional groups are excited to perform stretching-, deformation- and scissor-vibrations. In comparison to the MIR region, where only fundamental vibrations (“signatures”) can be observed, overtones and combinations can be found in the NIR region containing a manifold of information compared to MIR [9]. The result is often a crowded

![Figure 22.1 Electromagnetic spectrum. IR, infrared; NIR, near-infrared.](image-url)
spectrum with overlapping peaks. Although NIR-intensities are 10 to 100 times lower than for MIR, highly sensitive spectrometers can be built through several means including the use of efficient detectors. The light recorded by the detector contains compositional information, which can be unraveled by a computer to report multiple analyses almost instantaneously. NIR spectroscopy can provide simultaneous, rapid, and nondestructive qualitative and quantitative analysis of major components in many organic substances [10].

### 22.2.2 Model of the Harmonic and An-Harmonic Oscillator

The physical principle describing the observed effects in both the MIR and NIR regions is the model of the harmonic and an-harmonic oscillator. According to the inset in Figure 22.2, the reduced mass $\mu$ performs vibrations with the frequency $\nu_{osc}$. In MIR, this vibration follows the equation for the harmonic oscillator, whereas in NIR, the equation for the an-harmonic oscillator is valid, describing the excitation into higher energy states. Chemometrics, a mathematical, statistical, multivariate analytical (MVA) tool, is applied for further treatment of recorded spectra.

\[
\mu = \frac{mM}{m+M} \quad \text{reduced mass}
\]

\[
\nu_{osc} = \frac{k}{2\pi \sqrt{\mu}}
\]

**FIGURE 22.2** Model of the harmonic and an-harmonic oscillator.

### 22.2.3 Instrumentation and Sample Preparation

An NIR spectrophotometer consists of a light source (e.g., tungsten halogen lamp), sample presentation accessories, monochromator, detector, and optical components including lenses, collimators, beam splitters, integrating spheres, and optical fibers (Figure 22.3) [11].

One of the most frequently cited benefits of analysis by NIR spectroscopy is that little or no sample preparation prior to analysis is required. In principle, transparent materials such as liquid extracts can be analyzed by transmission or transflection, solid materials like tissue by diffusive reflection and/or interactance mode (Figure 22.4) [11]. Spectrophotometers are conveniently classified into dispersive and nondispersive instruments. For example, in a dispersive filter instrument, the monochromator is a wheel holding a number of absorption or interference filters, with the disadvantage of limited resolution [11]. In a scanning monochromator instrument, a grating or a prism is used to separate the individual frequencies of the radiation. In Fourier transform (FT) spectrophotometers, interferometers are used to generate modulated light, and time domain signal of the light reflected or transmitted by the sample can be converted into a spectrum.
via a fast transformation [12]. In most cases, a Michelson or polarization interferometer is used.

Photodiode array (PDA) spectrophotometers consisting of a fixed grating that focuses the dispersed radiation onto an array of silicon (Si, 350–1100 nm) or indium gallium arsenide (InGaAs, 1100–2500 nm) offer the advantage of high acquisition speed (between 50 ms and a few milliseconds). As alternatives, laser-based systems that do not require a monochromator or acousto-optic tuneable filter instruments using a diffraction-based optical band-pass filter can be used [13].

**22.3 IR IMAGING SPECTROSCOPY**

The first IR microscopes were built during the 1940s and 1950s, but these microscopes were slow. It was in the 1990s that the subsequent development of microprocessor-controlled motorized stages made raster scan mapping convenient. Enhanced spatial resolution, which is enabled by the substitution of synchrotron radiation for a thermal source, was a significant instrumental advance. The introduction of focal plane array (FPA) systems, initially uncooled InGaAs, for near-IR by Mascott and Lewis [14] occurred in 1994, while the subsequent development for mid-IR, using a mercury cadmium telluride (MCT) array by Lewis and Levine [15] resulted in a very rapid image generation. These developments allowed simultaneous measurements and significantly increased data acquisition rates.

**22.3.1 Imaging Microscopy**

Fourier transform IR (FTIR) spectroscopic microscopy can be considered as the coupling of a microscope to an IR spectrometer. From the physical point of view, diffraction, refraction, reflection, and absorption effects are playing a considerable role. In principle, an FTIR microscope is similar to its optical analog, and consists of four main parts: (1) light source (single polychromatic thermal source), (2) splitter ((FT), tunable filter and diffraction grating), (3) detector (photon detectors, lead sulfide (PbS) detectors, indium antimonide (InSb) detectors, uncooled InGaAs and HgCdTe or MCT detectors), and (4) optics (fitted to a microscope) [16] (Figure 22.5). Advantages of FTIR spectroscopic microscopes are microspatial chemical mapping or imaging of complex heterogeneous samples with a resolution down to approximately 1.2 μm, high sensitivity, high selectivity, rapid data acquisition, simple sample preparation, fully automated examination, and computer-enhanced visualization.

**22.3.2 Measurement Techniques**

During the last couple of years, hyperspectral imaging systems have become popular [16]. A multispectral
(a few wavelengths) or hyperspectral (a continuous range of wavelengths) cube is recorded, consisting of spectra recorded at every 2-D spatial position (Figure 22.6). The cube is recorded by stepwise moving of the object of interest under the camera by means of an actuator, while at each step a line is scanned. In latest developments, FPA detectors are employed (MCT). In combination with attenuated total reflection (ATR) spectroscopy, the maximal resolution of 1.2 μm can be reached [10,16].

In quantitative analysis, the amount of absorbed radiation is dependent upon the Lambert–Beer law of the concentration $c$ of the sample, the thickness $d$ of the sample, and its molar extinction coefficient $\varepsilon$.

$$ E = \log \frac{I_0}{I} = \varepsilon * d * c $$

$I$ intensity of the transmitted light $I_0$ intensity of the incident light $c$ concentration of the absorbing substance (unit: mol/dm$^3$ or mol/L) $\varepsilon$ decimal extinction coefficient (unit: mol$^{-1}$.dm$^2$) $d$ thickness of the irradiated body (unit: cm)

### 22.3.3 Data Recording

There are two possible experimental measurement set-ups in FTIR microscopy: FTIR mapping and FTIR imaging. In FTIR mapping, the IR spectra of the sample
are collected sequentially at predefined spatial coordinates. This sampling technique offers a convenient, fast, and inexpensive route for analysis of static samples. It allows information about the spatial distribution of the chemical species within the sample to be obtained. The operator can choose between three different types of FTIR mapping techniques:

1. **Point mapping** provides several different areas of a sample to be analyzed consecutively.
2. **Line mapping** defines a series of spectra obtained along one dimension, where chemical changes that occur along this dimension are investigated.
3. **Area mapping** defines a series of spectra to be collected in two dimensions.

In FTIR imaging, the whole area of interest is sampled simultaneously and allows a large number of spectra to be acquired with fine spatial detail over an area [17]. However, obtaining imaging data are challenging, and a key issue is how to extract relevant information from the huge amount of data. In FTIR microscopy, one can choose between two different sampling techniques: transmission (IR beam passes through the sample) and reflection (IR beam reflects from the sample surface). In transmission measurements, the IR beam passes through the sample and the transmitted light is recorded by a detector. In reflection measurements, the incoming radiation interacts with the sample and is scattered by interaction with the particles. A fraction of this light is reflected by the sample and recorded by the detector. In the NIR range, DR is widely used for the image analysis of thick nontransparent samples in various noninvasive applications (e.g., food industry and pharmaceuticals) [19].

In ATR measurements, the IR radiation enters a prism made of a high refractive index IR transmitting material, and is totally internally reflected. This reflectance creates an evanescent wave. The wave extends beyond the surface of the crystal into the sample held in intimate contact with the crystal and in regions of the IR spectrum where the sample absorbs energy. With this sampling technique, the IR beam typically penetrates from 0.5 to 2.0 µm into the sample. Main advantages of ATR imaging measurements are minimal or no sample preparation. Additionally, samples with high water contents can be analyzed more efficiently than in conventional transmission mode. For detailed information about detector theory, technology, and current developments, the interested reader is referred to the cited literature.

Data processing techniques for imaging data will be discussed in the next chapter.
22.4 CHEMOMETRICS INCLUDING DATA PREPROCESSING

22.4.1 Chemometrics in NIR Spectroscopy

The NIR spectrum is represented by a huge number of partially overlapping overtones and combination vibrations, therefore appears to be much more complicated than the MIR spectrum. Additionally, scattering effects, instrumental noise, and/or sample inhomogeneities can occur. Therefore, it is in many cases impossible to correctly assign the corresponding vibration bands. For this reason, multivariate statistical analysis (MVA) is a powerful mathematical tool enabling the extraction of the required information from the spectrum [20].

The most frequently applied chemometrical procedures include principal component analysis (PCA) for reducing the number of variables facilitating both qualitative and quantitative analysis. Data pretreatment minimizes inhomogeneities originating from the recording of the spectra, and enables elimination of baseline shifts. Normalization algorithms can eliminate differences in intensity caused by different sample positioning. Diffusion and/or unexpected particle size effects can be compensated by multiplicative scatter correction (MSC). Performing the first or second derivative of the original spectrum can reduce spectral noise. Calibration development can mathematically describe the covariation between certain variables or find a mathematical function (regression model) by which the values of the dependent variables are calculated from values of the measured variables [21]. The calibration procedure of the NIR spectrometer can be summarized in five steps: (1) choice of a representative sample set; (2) recording of the NIR spectra; (3) measurement of the reference values; (4) multivariate modelling to generate a relationship between the recorded spectra and the reference values; and (5) validation of the system. The most frequently used regression methods comprise principal component regression (PCR) and partial least squares regression (PLSR), discriminant analysis (DA) and artificial neural networks (ANN) [6]. The choice of the highest suitable regression model is based on the calculation of the following values:

1. BIAS, i.e., the average deviation between the predicted values ($y_n$) and the actual values ($x_n$), in the calibration set, should be close to zero.

   \[
   \text{BIAS} = \frac{1}{N} \sum (x_n - y_n) \tag{22.2}
   \]

2. PRESS (predicted residual error sum square) is the sum of the square of the deviation between predicted and reference values. The PRESS value of the validation set should be as small as possible and similar to that of the calibration set.

   \[
   \text{PRESS} = \sum (x_n - y_n)^2 \tag{22.3}
   \]

3. Standard error of estimation (SEE), i.e., the standard deviation of the differences between reference values and NIRS results in the calibration set.

   \[
   \text{SEE} = \sqrt{\frac{1}{N} \sum (x_n - y_n - \text{BIAS})^2} \tag{22.4}
   \]

4. Standard error of prediction (SEP), i.e., the counterpart for the test set samples. SEE and SEP should be as small as possible.

   \[
   \text{SEP} = \sqrt{\frac{1}{N} \sum (x_n - y_n - \text{BIAS})^2} \tag{22.5}
   \]

5. The correlation coefficient ($R^2$) should approach 1.

The analysis of IR imaging data sets includes denoising, baseline correction, normalization, suppression of anomalous pixels, image compression, and univariate and multivariate analysis [16]. Available software programs improve the quality of information extracted from the large data sets, reduce the dimensionality to more practical levels, allow different imaging data sets to be aligned and compared, and address data management, including determination of statistical significance and relative abundance between particular chemical species. It has been reported that data analysis of IR imaging results consists of several steps:

- Demonstration of a constituent by taking a slice of the image on a particular relevant wavenumber, which gives selective information for the particular compound. Resulting distribution images can be easily interpreted with minimal computation effort and allow the observation of certain spatial features in the image. This simple method (known as univariate analysis) can only provide partial representation of the obtained imaging data and likely makes comparisons of several data sets impossible [18].
- Reduction of the complexity of IR imaging datasets with PCA, which transforms the original coordinate system defined by peak intensities to a coordinate system that better explains the variance in the dataset.
- Unsupervised classification such as hierarchical clustering, k-means (KM) clustering, and fuzzy C-means (FCM) clustering use all of the information contained in the hyperspectral image, where unlabeled IR spectral data can be separated into different clusters based on their characteristics in an unsupervised way.
22.4.2 Chemometrics in Imaging Spectroscopy

To analyze the spectral and spatial information contained in an image, various techniques also known as multivariate image analysis (MIA) have been introduced. In contrast to univariate image analysis, MIA uses all of the information contained in the hyperspectral image. Unlabeled IR spectral data can be separated into different clusters based on their characteristics in an unsupervised way. Clustering, also called unsupervised classification of FTIR microscopic data, can be performed such that spectra within the same cluster are as similar as possible and spectra in different clusters are as dissimilar as possible where different types of cells may be separated within biological tissue. There are many clustering techniques that have been applied for hyperspectral images, such as hierarchical clustering, KM clustering, and FCM clustering, which increase the information content of FTIR imaging data dramatically [18]. For detailed information about statistical classification, the interested reader is referred to the cited literature.

22.5 AD- AND DISADVANTAGES OF NIR AND IMAGING SPECTROSCOPY

The ad- and disadvantages of NIR spectroscopy are summarized in the following.

Advantages:
- Noninvasive measurement (sample can be used for other purposes after the measurement)
- Simultaneous determination of several parameters (the information is all packed within the spectrum)
- Chemical and physical properties can be determined in parallel (e.g., ingredients and solvent composition)

Disadvantages:
- Calibration takes a long time, requiring the consultancy of a reference method
- The lower limit of detection (LOD) can normally be found in the lower percentage range

The advantages and disadvantages of imaging spectroscopy are summarized in the following.

Advantages:
- Noninvasive measurement
- Huge potential for spectral interpretation
- Sensitive

Disadvantages:
- Time consuming (measurement takes several hours)
- Complicated sample preparation before measurement

22.6 QUANTITATIVE ANALYSIS OF SECONDARY METABOLITES

In this chapter, we discuss the potential of NIR spectroscopy for the quantitative characterization of herbal medicine and its constituents, including secondary metabolites and leading compounds. The following section summarizes the potential of NIR spectroscopy for the classification of the origin of natural products and verification of authenticity.

22.6.1 Phenolic Compounds

NIR spectroscopy has been applied to determine the content of total polyphenols, catechins, and others [22, 23]. Furthermore, attempts to analyze the antioxidative [24], antimicrobial [25], antiviral [26], anti-inflammatory, analgesic, antipyretic, and vasodilatory effects have been described [27].

Green tea: The quantitative analysis of the epigallocatechin (EGC) gallate, epicatechin (EC), and trolox equivalent antioxidant capacity (TEAC) in green tea (Camellia sinensis L.) leaves was described. As a reference for the control of the total phenolic content in green tea, Folin-Ciocalteu (FC) was used, resulting in an RMSECV of 0.75 g/g for a calibration range of 15.84–24.39 g/g [28]. For the quantitation of total polyphenol content high-performance liquid chromatography (HPLC) was applied. Chen and coworkers compared the three algorithms partial least square (PLS), interval PLS (iPLS), and synergy interval PLS (siPLS) to predict the total polyphenol content [29]. The siPLS model performed best, with an RMSEP of 0.7327 (range: 15.84–24.39%) using five PLS factors. The same work group reported PLS calibrations to determine the contents of the main catechins. Using 75 samples, EGC (0.14%, range: 2.4–5.4%), EC (0.017%, range: 0.1–0.4%), epigallocatechingallate (EGCG) (0.38%, range: 7.7–14.1%), and epicatechingallate (ECG) (0.12%, range: 1.8–3.7%) were calibrated using 10–14 PLS factors. Luypaert et al. [30] reported on a NIR spectroscopy method in combination with PLS algorithms to predict caffeine, EGCG, EC, and total antioxidant capacity. Zhang and coworkers [31] predict the total antioxidant capacity in green tea using PCR regression with test set validation, with 100 samples in the calibration set and 23 in the validation set.

Grape skins: Phenolic compounds in grape skins and intact grapes during ripening were analyzed by Ferrer-Gellego et al. [32]. Anthocyanins, phenolic acids, flavonols, flavanols, and total phenolic compounds were quantified with standard ratio of performance to deviations (RPDs) ranging from 4.4–13.6 applying partial least square (PLS) (sample set of 56). The same workgroup also worked on calibration models to determine flavanols in grape seeds [33].
**Blueberries**: Total phenols, total flavonoids, total anthocyanins, and ascorbate in blueberries (*Vaccinium corymbosum*) were investigated using a system to determine total soluble solid (TSS), applying NIR and MIR spectroscopy comparing cross validation and test set validation, respectively [34].

**Kava**: In the South Pacific, kava (*Piper methysticum*) has been traditionally used for thousands of years for relaxation without the loss of mental alertness. Kava has become a part of the herbal pharmacopoeia throughout the United States and Europe because of its anxiolytic properties [35]. A PLS calibration model for kavalactones (desmethoxyyangonin, dihydrokavain, yangonin, kavain, dihydromethysticin, methysticin and total kalvalactones) using a maximum of 7 PLS factors was developed by Gautz and coworkers [35]. The SEPs were 0.20% (range: 0.08 e 2.35%) for desmethoxyyangonin, 0.31% (range: 0.10 e 3.33%) for dihydrokavain, 0.47% (range: 0.08 e 3.02%) for yangonin, 0.21% (range: 0.11 e 3.02%) for kavain, 0.15% (range: 0.08 e 2.58%) for dihydromethysticin, 0.19% (range: 0.09 e 2.70%) for methysticin and 1.05% (range: 0.54 e 14.68%) for total kalvalactones.

**Honeybush**: Mangiferin and hesperidin in dried green honeybush (*Cyclopia genistoides*) were analyzed by Joubert et al. [36]. PLS calibrations were calculated using 160 samples with four and six PLS factors, resulting in SEPs of 0.46% (range: 0.70 e 7.21%) for mangiferin and 0.38% (range: 0.64 e 4.80%) for hesperidin. RPDs were 1.96 (mangiferin) and 1.90 (hesperidin).

**Rooibos**: An NIR spectroscopy method in combination with PLS to predict total polyphenols, aspalathin, nothofagin, and dihydrochaclone in dried green rooibos (*Aspalathus linearis*) and aspalathin in water extracts was reported by Manley et al. [37]. The addition of dried rooibos extract powder to some of the samples helped to increase the aspalathin and nothofagin content.

**Magnolia officinalis**: An NIR spectroscopy method for quantification of phenolic compounds in *M. officinalis* was reported by Yu and coworkers [38]. PLS, mPLS, and PCR algorithms were compared, with mPLS performing best, with reaching correlation coefficients of 0.97 for the calibration and 0.95 for validation.

**Primula**: A method for controlling the flavonoid content in *Primulae veris flos* was developed by Huck et al. This herbal medicine is used as an expectorant related to its anti-inflammatory properties for the treatment of sinusitis. For the control of the *Primulae veris flos* content, the leading compound 3,4,5-trimethoxyflavone was determined by reversed-phase liquid chromatography (RP-LC) as a reference method. The ethanol/water ratio was controlled simultaneously with the same system with a correlation coefficient of 0.99,530 for water (reference method: Karl-Fischer titration), and a coefficient of 0.99,701 for ethanol (reference method: GC). Validation and results of real samples showed that the robustness and reproducibility of the NIR spectroscopy model for the determination of the 3,4,5-trimethoxyflavone, water, and ethanol content is high (Figure 22.7) [39]. For the identification of *Primulae veris flos* and quantitation of the leading compound, NIR spectroscopy was applied as a detector in TLC [39].

Schoenbichler et al. described a study using NIR and attenuated total-reflectance IR (ATR-IR) spectroscopy in hyphenation with PLSR to determine the antioxidant

![Graph showing the relationship between predicted value of NIR and true value of HPLC.](image)

**FIGURE 22.7** Quantitative analysis of 3,4,5-trimethoxyflavone, water, and ethanol content in a liquid plant extract. SEE, standard error of estimation; SEP, standard error of prediction.
capacity of *Primulae flos cum calycibus* samples [39].

FC, ferric ion reducing antioxidant power (FRAP), 2,2-diphenyl-picrylhydrazyl (DPPH), 2,2’-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diaminonum salt (ABTS), and cupric reducing antioxidant capacity (CUPRAC) assays were performed as reference methods. Different spectra pretreatments such as standard normal variate (SNV), first or second derivative were applied to remove scattering effects. The ability of the two spectroscopic techniques to replace the five assays was evaluated and compared. In general, NIR demonstrated advantages over ATR-IR spectroscopy, and had the best results for the ABTS assay (R²: 0.94, RPDcorr: 4.66; test set validation). Also, ATR-IR spectroscopy revealed the best prediction power for the ABTS assay (R²: 0.94, RPDcorr: 4.10; test set validation). The feasibility of vibrational spectroscopy as a fast and simple tool to replace wet chemistry assays for the measurement of the antioxidant capacity of *Primulae flos cum calycibus* samples was demonstrated [39].

**St. John's wort:** St. John’s wort extract is used for treatment of skin injuries, burns, neuralgia, for its bacteriostatic and bactericide activity, and as a treatment for mild to moderate depression. The pharmacological mechanism by which this extract works as an antidepressant is still not discovered. Both hypericin and hyperforin play an important role as standards in the phytopharmaceutical industry. For the investigation of St. John’s Wort and its ingredients, different analytical procedures have been established, including IR imaging spectroscopy, UV spectroscopy, fluorescence microscopy, TLC, LC, LC coupled with mass spectrometry (LC-MS), and CE. Prior to spectroscopic analysis via NIR spectroscopy, a reference method based on LC, LC-MS, and CE was established [40]. In the following, 320 spectra of 80 extracts were recorded in transfection mode using light fiber optics over a wavelength range from 4008 to 9996/cm with a resolution of 1 mm. The most intensive band in the first derivative spectrum belonged to the vibration of the second overtone of the carbonyl group (5352/cm), followed by C=H stretch and C–H deformation vibration, the –OH vibration (4440/cm) and the –CH₂ overtone (5742/cm). Five primary factors were necessary to reach the best calibration equation. The robustness of the established NIR spectroscopy model is high, which is demonstrated in the similarity of results for SEE and SEP: 0.52 and 0.50 μg/mL and 0.64 and 0.71 μg/mL for hypericin and hyperforin, respectively (Table 22.1).

**Rice grain:** Total phenolic and flavonoid contents as well as antioxidant capacity of rice grain was discussed by Zhang et al. applying NIR spectroscopy in combination with multivariate data analysis [41]. The PLS and mPLS algorithms were compared, delivering similar low SEP and correlation coefficients above 0.84 for total phenolics and antioxidant capacity.

**Honghua oil:** Wu and coworkers [42] reported about MIR and NIR spectroscopic methods for quantitative analysis of the three marker components, α-pinene, methyl salicylate, and eugenol, to assess the quality of honghua oil, a traditional Chinese medicine (TCM) oil preparation, consisting of several plant essential oils.

**Bamboo leaves:** Flavonoids and phenolic acids in extracts of bamboo leaves were found by Lu et al. [43]. NIR spectroscopy was combined with PLS and least-squares support vector machine (SVM) to establish the corresponding models.

**Snow lotus:** Chen and coworkers [44] applied NIR spectroscopy to measure the total flavone content in snow lotus (*Saussurea involucrata*) using iPLS with genetic algorithm (iPLS-GA).

**Alfalfa:** Gonzalez-Martin [45] applied NIR spectroscopy to determine tocopherols in alfalfa. Using mPLS calibration models for 60 fresh and dehydrated samples, SECVs of 0.37 mg/100 g for α-tocopherol (range: 0.55–5.16) and 0.027 for (β + γ)-tocopherol (range: 0.07–0.48) were achieved.

**Radix puerariae:** Lau et al. [46] determined the puerarin, daidzin, and total flavonoids in *R. puerariae* by calculating PLS regression models. Correlation coefficients in the range 0.939–0.970 were reached using a maximum of five PLS factors.

**Ginkgo biloba:** Zhou and coworkers [47] reported about NIR spectroscopy in combination with iPLS to determine quercetin in extracts of *G. biloba*.

**Cannabis:** An NIR spectroscopy method to discriminate between tetrahydrocannabinol (THC)-rich and hemp forms of cannabis was reported by Wilson et al. [48].

**Scutellariae radix:** This plant, also known as huang-qin, is of high pharmacological interest due to the contained flavonoids. About 30 flavonoids were identified and quantified in *Radix scutellariae*, using different analytical techniques such as CE [49], gas chromatography, TLC, ion-pair HPLC, HPLC with UV spectroscopy, high-speed counter-current chromatography (HSCCC), HPLC, and HPLC coupled with MS [50]. The main flavonoids contained in the plant are: wogonin and baicalin; the latter was found in higher amount. Various scientific publications investigated several pharmacological effects of baicalin and its aglycone baicain as an antitumor agent [51] that can inhibit cancer cell growth or induce apoptosis in breast, prostatic cell lines, and act as anti-inflammatory, antioxidant, and free radical scavenger, as well as an antiviral (HIV), anti-SARS coronavirus agent [52]. Huang and coworkers [53] determined baicain and total flavonoids in Radix scutellariae measuring 61 samples in DR mode with...
| Pretreatments   | Hyperforin SEP [%] | Hyperforin PCs | Hyperforin r²(val.) | Hypericin SEP [%] | Hypericin PCs | Hypericin r²(val.) | Rutoside SEP [%] | Rutoside PCs | Rutoside r²(val.) | Hyperoside SEP [%] | Hyperoside PCs | Hyperoside r²(val.) | Hypertorin SEP [%] | Hypertorin PCs | Hypertorin r²(val.) | Hypericin r²(val.) |
|----------------|------------------|----------------|---------------------|------------------|----------------|-------------------|-----------------|-------------|-----------------|------------------|----------------|-------------------|------------------|----------------|------------------|------------------|
| Normalization | 1.29             | 0.00655        | 1.58                | 0.249            | 1.41           | 0.00418           | 0.976           | 0.141       |                 |                  |                 |                   |                  |                 |                   |
|               | 0.914            | 0.679          | 0.61                | 0.757            | 0.895          | 0.7               | 0.856           | 0.912       |                 |                  |                 |                   |                  |                 |                   |
|               | 2.75             | 1.58           | 1.57                | 1.96             | 2.52           | 2.47              | 2.54            | 3.46        |                 |                  |                 |                   |                  |                 |                   |
| SNV           | 1.24             | 0.00624        | 1.54                | 0.253            | 1.4            | 0.0035            | 0.935           | 0.133       |                 |                  |                 |                   |                  |                 |                   |
|               | 0.923            | 0.67           | 0.631               | 0.749            | 0.893          | 0.8               | 0.863           | 0.901       |                 |                  |                 |                   |                  |                 |                   |
|               | 2.87             | 1.66           | 1.61                | 1.93             | 2.54           | 2.95              | 2.66            | 3.67        |                 |                  |                 |                   |                  |                 |                   |
| MSG           | 1.25             | 0.00629        | 1.558               | 0.252            | 1.53           | 0.00343           | 0.976           | 0.186       |                 |                  |                 |                   |                  |                 |                   |
|               | 0.921            | 0.667          | 0.624               | 0.751            | 0.856          | 0.8               | 0.851           | 0.829       |                 |                  |                 |                   |                  |                 |                   |
|               | 2.84             | 1.64           | 1.59                | 1.94             | 2.32           | 3.01              | 2.54            | 2.62        |                 |                  |                 |                   |                  |                 |                   |
| 1st derivative| 1.12             | 0.00794        | 1.46                | 0.173            | 1.39           | 0.00328           | 1.55            | 0.145       |                 |                  |                 |                   |                  |                 |                   |
|               | 0.921            | 0.454          | 0.729               | 0.86             | 0.874          | 0.881             | 0.675           | 0.915       |                 |                  |                 |                   |                  |                 |                   |
|               | 3.17             | 1.30           | 1.70                | 2.82             | 2.56           | 3.15              | 1.60            | 3.37        |                 |                  |                 |                   |                  |                 |                   |
| 2nd derivative| 1.27             | 0.00695        | 1.29                | 0.169            | 1.51           | 0.00565           | 1.37            | 0.139       |                 |                  |                 |                   |                  |                 |                   |
|               | 0.91             | 0.692          | 0.771               | 0.887            | 0.87           | 0.754             | 0.706           | 0.926       |                 |                  |                 |                   |                  |                 |                   |
|               | 2.80             | 1.49           | 1.92                | 2.89             | 2.35           | 1.83              | 1.81            | 3.51        |                 |                  |                 |                   |                  |                 |                   |
| 3rd derivative| 1.50             | 0.00678        | 1.34                | 0.211            | 1.43           | 0.00506           | 1.38            | 0.115       |                 |                  |                 |                   |                  |                 |                   |
|               | 0.861            | 0.702          | 0.729               | 0.81             | 0.898          | 0.788             | 0.703           | 0.947       |                 |                  |                 |                   |                  |                 |                   |
|               | 2.37             | 1.52           | 1.85                | 2.31             | 2.49           | 2.04              | 1.80            | 4.25        |                 |                  |                 |                   |                  |                 |                   |
| Norm. + 1st der.| 1.30            | 0.00764        | 1.35                | 0.17             | 1.36           | 0.00402           | 1.47            | 0.164       |                 |                  |                 |                   |                  |                 |                   |
|               | 0.915            | 0.684          | 0.779               | 0.846            | 0.879          | 0.827             | 0.711           | 0.895       |                 |                  |                 |                   |                  |                 |                   |
|               | 2.73             | 1.35           | 1.84                | 2.87             | 2.61           | 2.57              | 1.69            | 2.98        |                 |                  |                 |                   |                  |                 |                   |
| SNV + 1st der. | 1.19             | 0.00762        | 1.35                | 1.51             | 1.50           | 0.00411           | 1.33            | 0.186       |                 |                  |                 |                   |                  |                 |                   |
|               | 0.917            | 0.676          | 0.779               | 0.866            | 0.857          | 0.763             | 0.718           | 0.865       |                 |                  |                 |                   |                  |                 |                   |
|               | 2.99             | 1.36           | 1.84                | 0.32             | 2.37           | 2.52              | 1.87            | 2.62        |                 |                  |                 |                   |                  |                 |                   |
| MSG + 1st der. | 1.19             | 0.00754        | 1.36                | 0.15             | 1.51           | 0.00411           | 1.32            | 0.185       |                 |                  |                 |                   |                  |                 |                   |

**Continued**
### TABLE 22.1 Summarized Quality Parameters of the Performed PLSR Calibrations for the Determination of St. John’s Wort Ingredients by NIR Compared to MIR-ATR Spectroscopy [61]—cont’d

| Pretreatments | Hyperforin | Hypericin | Rutoside | Hyperoside | Hyperforin | Hypericin | Rutoside | Hyperoside |
|---------------|------------|-----------|----------|------------|------------|-----------|----------|------------|
|               | SEP [%]    | SEP [%]   | SEP [%]  | SEP [%]    | SEP [%]    | SEP [%]   | SEP [%]  | SEP [%]    |
|               | PCs        | PCs       | PCs      | PCs        | PCs        | PCs       | PCs      | PCs        |
|               | r²(val.)   | r²(val.)  | r²(val.) | r²(val.)   | r²(val.)   | r²(val.)  | r²(val.) | r²(val.)   |
|               | RPD        | RPD       | RPD      | RPD        | RPD        | RPD       | RPD      | RPD        |
| Norm. + 2nd der. | 5          | 5         | 3        | 4          | 7          | 4         | 6        | 4          |
|               | 0.917      | 0.678     | 0.777    | 0.866      | 0.856      | 0.757     | 0.724    | 0.867      |
|               | 2.99       | 1.37      | 1.83     | 3.25       | 2.35       | 2.51      | 1.88     | 2.64       |
| SNV + 2nd der. | 1.36       | 0.00689   | 1.02     | 0.17       | 1.45       | 0.00765   | 1.33     | 0.168      |
|               | 6          | 4         | 6        | 4          | 4          | 2         | 6        | 3          |
|               | 0.921      | 0.708     | 0.925    | 0.879      | 0.865      | 0.528     | 0.718    | 0.898      |
|               | 2.61       | 1.50      | 2.43     | 2.87       | 2.45       | 1.35      | 1.87     | 2.91       |
| MSG + 2nd der. | 1.35       | 0.00689   | 0.979    | 0.159      | 1.58       | 0.00309   | 1.25     | 0.177      |
|               | 6          | 4         | 6        | 4          | 6          | 6         | 7        | 3          |
|               | 0.915      | 0.707     | 0.859    | 0.894      | 0.87       | 0.895     | 0.751    | 0.895      |
|               | 2.61       | 1.49      | 2.54     | 3.07       | 2.25       | 3.35      | 1.99     | 2.76       |
| Norm. + 3rd der. | 1.24       | 0.00602   | 1.16     | 0.157      | 1.46       | 0.00594   | 1.37     | 0.202      |
|               | 7          | 5         | 6        | 4          | 5          | 2         | 5        | 2          |
|               | 0.909      | 0.753     | 0.816    | 0.885      | 0.901      | 0.623     | 0.698    | 0.882      |
|               | 2.87       | 1.72      | 2.14     | 3.11       | 2.43       | 1.74      | 1.81     | 2.42       |
| SNV + 3rd der. | 1.21       | 0.00589   | 1.13     | 0.153      | 1.445      | 0.00411   | 1.33     | 0.122      |
|               | 7          | 5         | 6        | 4          | 5          | 6         | 6        | 4          |
|               | 0.909      | 0.763     | 0.815    | 0.892      | 0.902      | 0.813     | 0.722    | 0.947      |
|               | 2.94       | 1.75      | 2.20     | 3.19       | 2.46       | 2.51      | 1.87     | 4.00       |
| MSG + 3rd der. | 1.21       | 0.00585   | 1.14     | 0.151      | 1.45       | 0.00411   | 1.33     | 0.12       |
|               | 7          | 5         | 6        | 4          | 5          | 6         | 6        | 4          |
|               | 0.909      | 0.764     | 0.815    | 0.895      | 0.902      | 0.813     | 0.72     | 0.948      |
|               | 2.94       | 1.77      | 2.18     | 3.23       | 2.45       | 2.51      | 1.87     | 4.07       |

SEP, standard error of prediction; SNV, standard normal variate; NIR, near-infrared; MIR, mid-infrared; ATR, attenuated total reflection.
22.6.2 Glycoside Compounds

*Brassica*: Tartary buckwheat (*Fagopyrum tartaricum*) was analyzed by Yang and coworkers [54]. They established PLS regression models to quantify the rutin and D-chiro-inositol (DCI) content.

*Verbena officinalis*: ATR-IR and NIR diffuse reflectance spectroscopy (NIR) in hyphenation with multivariate analysis was used to quantify verbenalin and verbascoside in *V. officinalis* by Schoenbichler et al. [55]. A new HPLC method as a reference was established and validated, being highly suitable as a reference method for calibrating the IR models. For both, vibrational spectroscopic methods test set and cross validation were performed. Different data pretreatments like SNV, first and second derivative were applied to remove systematic errors and were evaluated. Quality parameters obtained for the test set validation revealed that ATR-IR (verbenalin: R² = 0.94, RPD = 4.23; verbascoside: R² = 0.93, RPD = 3.63) has advantages over NIR (verbenalin: R² = 0.91, RPD = 3.75; verbascoside: R² = 0.80, RPD = 2.35) in the given application.

22.6.3 Ginsenosides

Yap et al. performed simultaneous quantification of ginsenosides Rb1, Rb2, Rc, Re, Rd, Rg1, Ro, m-Rb1, m-Rb2, m-Rd, and m-Re in American ginseng. Among the calibration equations for the 11 individual ginsenosides, those of RB1, Re, and m-Rb1 showed the lowest relative standard deviation using HPLC as a reference method [56].

22.6.3.1 Glucosinolates

*Indian mustard*: 2700 winter Indian mustard seeds were analyzed using MPLS as regression method with reported SEP values of 15.65 for glucosinolates [57].

*Brassica*: The determination of glucosinolates in *Brassica* species [22], including *Brassica napus* L. [58], *pabularia*, *oleracea*, and *juncea* was reported.

22.6.3.2 Essential Oils

*Honghua oil*: Honghua oil, a TCM oil preparation, is a mixture of several plant essential oils. Gas chromatographic (GC) investigation of 48 commercially available oils was carried out to establish PLS calibrations for the three marker components *α*-pinene, methyl salicylate, and eugenol with SEP values of 1.55, 0.957, and 0.389%, respectively [42].

NIR spectroscopy shows great potential to use the generated fingerprint spectrum for classification, discrimination, and/or authentication queries [59]. Furthermore, it shows high potential for classifying the origin of natural products and detecting adulteration.

*Tea plant*: 293 samples from field experiments were analyzed by Xiaoli and Yong [60] and in the following wavelet transformation (WT), PCA and ANN were used to classify the tea samples. The ANN models developed gave good classification accuracy up to 77.3% for the varieties analyzed.

For the fast analytical judgment of green, black, and oolong tea, the combination of NIR spectroscopy with SVM was reported [29]. In this attempt, spectral features of each category can be used to differentiate in the NIR region the three tea varieties. The best classification rates were up to 90, 100, and 93.33%, using the calibration set and 90, 100, and 95% using the validation set, respectively.

*St. John’s wort*: Huck-Pezzei et al. [61] established a procedure to discriminate between pharmaceutical formulations containing either *Hypericum perforatum* or *Hypericum hirsutum* originating from China.

*Scutellariae radix*: The suitability of NIR to distinguish between 27 cultivated and 22 wild samples collected from nine different regions in China was investigated. Spectral differences between wild and cultivated plants were enhanced after second derivative preprocessing. The most intense band in each spectrum could be assigned to the second overtone of the carbonyl group (5352/cm), followed by the CH stretch and CH deformation vibration (7212/cm), the OH vibration (4440/cm), and the CH overtone (5742/cm). Second derivative preprocessing were enhanced after second derivative preprocessing. The most intense band in each spectrum could be assigned to the second overtone of the carbonyl group (5352/cm), followed by the CH stretch and CH deformation vibration (7212/cm), the OH vibration (4440/cm), and the CH overtone (5742/cm). Furthermore, PCA was used to develop a cluster model for qualitative analysis of wild and cultivated Radix scutellariae (not shown). The total variance explained by the first principal component was 81%. In this study, the best results were obtained when models were based on the 4200–7716/cm spectral region. Second derivative is obviously superior to other pretreatments.

*Tanreqing*: Tanreqing injection is a widely used patent drug in China. It is made from five kinds of TCM extracts, namely: Radix Scutellariae, *Forsythia suspense*, *Flos lonicerae*, bear gall powder, and *Cornu goralis*. It was used chiefly in treating infection of the upper respiratory tract and serious influenza, and also has satisfactory efficacy in treating severe acute respiratory syndrome (SARS) and A/H1N1 flu. In its manufacturing process, several kinds of intermediates need to be analyzed to ensure that the operation runs steadily. A qualitative model to monitor the quality of
produced batches according to process analytical technology (PAT) guidelines recommended by the United States Food and Drug Administration (FDA) was established by Wenlong et al. [62].

Saffron: An NIR spectroscopy method to determine the chemical composition and geographical origin of 111 samples from the main producing countries, Iran, Greece, and Spain was reported by Zalacain [63]. Compared to UV-VIS and HPLC as reference methods, NIR spectroscopy was found to be superior, following the ISO 3632 Technical Specification Normative.

22.8 IR IMAGING SPECTROSCOPY STUDIES

The goals of plant IR spectroscopic imaging are to determine the severity of plant diseases, detect defects and contaminations, and determine the distribution of certain chemical components such as cellulose, hemicelluloses, lignin, lipids, proteins, and DNA [64]. An approach to understanding the function and the biochemical composition of plant tissue is one aim of imaging studies. IR spectroscopic imaging and mapping technologies have proven to be powerful options for generating new information on plant tissue compositions and alterations. The use of FTIR imaging to evaluate differences in the chemical composition of the Urtica dioica root tissue was described by Pallua et al. [64]. Tissue specimens were cut on a microtome at 5-μm thickness and mounted onto CaF2-slides. FTIR microscopic measurements were performed on an FTIR microscope equipped with a liquid nitrogen-cooled MCT 16-element linear array detector at atmospheric conditions. The samples were measured in MIR transmission mode with a nominal lateral resolution of 25 × 25 μm per pixel for each spot. Absorbance spectra were recorded in the range from 4,000/cm to 750/cm with a spectral resolution of 4/cm with two co-added scans at 1.0 cm/s step size. After measurement, the samples were analyzed by spectra, univariate, and cluster analyses. The analyses were performed using Spectrum software (PerkinElmer, England), Spectrum IMAGE software (PerkinElmer, England) and CytoSpec software package (http://www.cytospec.com, Germany). For the interpretation and calibration of the system, the FTIR-images were correlated with the histomorphological information. With this method, it was shown that it is possible to image different types of ingredients in the root applying a resolution of 25 × 25 μm.

Results presented in Figure 22.8 clearly illustrate the capability of spectroscopic imaging to accurately reproduce tissue histology of U. dioica root tissue. The optical image shows different tissue types: plerom tissue, periblem tissue, and rhizoderm tissue. The chemical map of the absorption at 1662–1645/cm, which is commonly attributed to amid-I proteins, represents a homogeneous distribution in all tissue types. This FTIR imaging result clearly demonstrated a very high absorption of this band in the periblem and rhizodermis.

FIGURE 22.8 IR imaging results of Urtica dioica samples. IR, infrared.
region, and indicates that these tissue types produce a high amount of amid-II proteins. A chemical map was generated by integrating the area under the band absorption at 1138–988/cm, which is commonly attributed to carbohydrates, nucleic acids, and phospholipids. The chemical map of the absorption at 1160–1140/cm correlated well with the morphology of the plerom region, and indicates that this tissue type produces a high amount of the mentioned ingredients. These observations led to the fact that the plerom tissue produces a high amount of carbohydrates, nucleic acids, and phospholipids compared to periblum or rhizoderm tissue. However, this form of processing cannot do specific correlations with morphological and histological features. Therefore, different cluster analyses are performed to fully characterize the range of spectral variations through the tissue section. Therefore, FCM and KM clustering are the most appropriate methods of choice.

22.9 REGULATORY ISSUES

In 2012, the European Medicine Agency published guidelines on the use of NIR spectroscopy by the pharmaceutical industry and the data requirements for new submissions and variations (EMEA/CHMP/CVMP/QWP/17,760/2009 Rev2; http://www.emeuropa.eu/docs/en_GB/document_library/Scientific_guideline/2012/02/WC500122769.pdf). This guideline describes the regulatory requirements for marketing authorization applications and variation applications submitted for medicinal products for human or veterinary use, which include the use of NIR spectroscopy. NIR spectroscopy is described in the European Pharmacopoeia; however, a single reference to the Ph.Eur. general chapter on NIR spectroscopy (Ph.Eur. 2.2.40) as a sole description for the NIR spectroscopic procedure is insufficient to support the use of such a procedure in marketing authorization applications or variation submissions.

This guideline outlines the requirements for applications in which NIR spectroscopy is used for qualitative and quantitative analysis or where it is used as a PAT for monitoring and controlling drug substance synthesis and finished product manufacturing processes.

Acknowledgments

The authors are grateful to Eurasia-Pacific Uninet (EPU) (Salzburg, Austria), the Ministry for Science and Research and the Ministry for Health, Family and Youth (Vienna, Austria) (Novel Analytical Tools for Quality Control in Traditional Chinese Medicine, Project No. 80855) for financial support.

References

[1] Krüger H, Schulz H. Analytical techniques for medicinal and aromatic plants. Stewart Postharvest Rev 2007;3:12.
[2] Schulz H. Analysis of coffee, Tea, Cocoa, Tobacco, Spices, medicinal and aromatic plants, and related products. Roberts CW[RJ], editor. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America; 2004.
[3] Stecher G, Huck CW, Stüggel WM, Bonn GK. Phytoanalysis: a challenge in phytomics. TrAC Trends Anal Chem 2003;22:1–14.
[4] Williams PC, Norris KH, Sobering DC. Determination of protein and moisture in wheat and barley by near-infrared transmission. J Agric Food Chem 1985;33:239–44.
[5] Blanco M, Coello J, Iturriaga H, Maspoch S, de la Puezuela C. Near-infrared spectroscopy in the pharmaceutical industry. Analyst 1998;123:135R–50R.
[6] Pallua JD, Recheis W, Pöder R, Pfliker K, Pezzei C, Hahn H, et al. Morphological and tissue characterization of the medicinal fungus Hericium coralloides by a structural and molecular imaging platform. Analyst 2011;137:1584–95.
[7] McClure WF. 204 years of near infrared technology: 1800–2003. J Near infrared Spectrosc 2003;11:487–518.
[8] Herschel W. Experiments on the refrangibility of the invisible rays of the Sun. Philes Trans R Soc London 1800;90:284–92.
[9] Barton F. Theory and principles of near infrared spectroscopy. Spectrosc Eur 2002;14:12–8.
[10] Osborne BG, Fearn T, Hindle PH. Practical NIR spectroscopy with applications in food and beverage analysis. Longman Scientific and Technical, 1993.
[11] Williams P, Norris K. Near-infrared technology in the agricultural and food industries. American Association of Cereal Chemists, Inc.; 1987.
[12] Faix O. In: Lin SY, Dence CW, editors. Methods in lignin chemistry. Berlin, Heidelberg: Springer Berlin Heidelberg; 1992.
[13] Nicoia BM, Beullens K, Bobelyn E, Peirs A, Saeys W, Theron KI, et al. Nondestructive measurement of fruit and vegetable quality by means of NIR spectroscopy: a review. Postharvest Biol Technol 2007;46:99–118.
[14] Lewis EN, Treado PJ, Reeder RC, Story GM, Dowrey AE, Marcott C, et al. Fourier transform spectroscopic imaging using an infrared focal-plane array detector. Anal Chem 1995;67:3377–81.
[15] Lewis EN, Levin IW. Real-time, mid-infrared spectroscopic imaging microscopy using indium antimonide focal-plane array detection. Appl Spectrosc 1995;49:672–8.
[16] Salzer R, Siesler HW. Infrared and Raman spectroscopic imaging. Wiley-VCH, 2009.
[17] Pezzei C, Pallua JD, Schaefer G, Seifarth C, Huck-Pezei V, Bittner LK, et al. Characterization of normal and malignant prostate tissue by Fourier transform infrared microspectroscopy. Mol Biosyst 2010;6:2287–95.
[18] Pallua DJ, Pezzei C, Schaefer G, Zelger B, Brunner A, Kloss-Brandstaetter A, et al. Advanced vibrational spectroscopic imaging of human tissue in life science. Curr Proteomics 2012;9:11.
[19] Gowen A, Odonell C, Cullen P, Downey G, Frias J. Hyperspectral imaging—an emerging process analytical tool for food quality and safety control. Trends Food Sci Technol 2007;18:590–8.
[20] Blanco M, Villarroya I. NIR spectroscopy: a rapid-response analytical tool. TrAC Trends Anal Chem 2002;21:240–50.
[21] Siebert KJ. Chemometrics in brewing—a review. J Am Soc Brew Chem 2001;59:147–56.
[22] Cozzolino D. Near infrared spectroscopy in natural products analysis. Planta Med 2009;75:746–56.
[23] Bittner L, Schönbiglsher S, Bonn G, Huck C. Near infrared spectroscopy (NIRS) as a tool to analyze phenolic compounds in plants. Curr Anal Chem 2013;9:417–23.
[24] Perron NR, Brumaghim JL. A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. Cell Biochem Biophys 2009;53:75–100.
[25] Rauha J-P, Remes S, Heinonen M, Hopia A, Kähkönen M, Kujala T, et al. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. Int J Food Microbiol 2000;56:3–12.
[26] Vijayan P, Raghu C, Ashok G, Dhanaraj SA, Suresh B. Antiviral activity of medicinal plants of Nilgiris. Indian J Med Res 2004;120:24–9.
[27] Padilla E, Ruiz E, Redondo S, Gordillo-Moscoso A, Slowing K, Tejerina T. Relationship between vasodilation capacity and phenolic content of Spanish wines. Eur J Pharmocol 2005;517:84–91.
[28] Quansheng C, Jiewen Z, Muhua L, Jianrong C, Jianhua L. Determination of total polyphenols content in green tea by near-infrared spectroscopy and multivariate calibration. Afr J Biotechnol 2011;10:8448–845.
[29] Chen Q, Zhao J, Fang CH, Wang D. Feasibility study on identification of green, black and Oolong teas using near-infrared reflectance spectroscopy based on support vector machine (SVM). Spectrochim Acta Part A 2007;66:568–74.
[30] Luyuapert J, Zhang MH, Massart DL. Feasibility study for the use of near infrared spectroscopy in the qualitative and quantitative analysis of green tea, Camellia sinensis (L.). Anal Chim Acta 2003;478:303–12.
[31] Zhang MH, Luyuapert J, Fernández Piernia JA, Xu QS, Massart DL. Determination of total antioxidant capacity in green tea by near-infrared spectroscopy and multivariate calibration. Talanta 2004: 62:25–35.
[32] Ferrer-Gallego R, Hernández-Hierro JM, Rivas-Gonzalo JC, Escrribano-Bailón MT. Determination of phenolic compounds of grape skins during ripening by NIR spectroscopy. LWT – Food Sci Technol 2011;44:847–53.
[33] Ferrer-Gallego R, Hernández-Hierro JM, Rivas-Gonzalo JC, Escrribano-Bailón MT. Feasibility study on the use of near infrared spectroscopy to determine flavonoids in grape seeds. Talanta 2010; 82:1778–83.
[34] Sinelli N, Spinardi A, Di Egidio V, Mignani I, Casiraghi E. Evaluation of quality and nutraceutical content of blueberries (Vaccinium corymbosum L.) by near and mid-infrared spectroscopy. J Agric Food Chem 2008;56:31–6.
[35] Gautz LD, Kaufusi P, Jackson MC, Bittenbender HC, Tang C-S. Determination of kavalactones in dried kava (Piper methysticum) powder using near-infrared reflectance spectroscopy and partial least-squares regression. J Agric Food Chem 2006;54:6147–52.
[36] Joubert E, Manley M, Botha M. Use of NIRS for quantification of mangiferin and hesperidin contents of dried green honeybush (Cyclopia genistoides) plant material. J Agric Food Chem 2006;54:5279–83.
[37] Joubert E, Manley M, Botha M. Evaluation of spectrophotometric methods for screening of green rooibos (Aspalathus linearis) and green honeybush (Cyclopia genistoides) extracts for high levels of Bio-active compounds. Phytochem Anal 2008;19:169–78.
[38] Yu C-Y. Quantification of phenolic compound in Magnolia officinalis herb by near infrared reflectance spectroscopy. J Zhejiang For Coll 2007;05.
[39] Schönbiglsher S, Bittner LKH, Pallua JD, Popp M, Abel G, Bonn GK, et al. Simultaneous quantification of verbena in and baicalein in human hepatoma cell lines. Cancer Lett 1994;86:91–5.
[40] Huck CW, Abel G, Popp M, Bonn GK. Comparative analysis of naphthodianthrene and phloroglucine derivatives in St. John’s Wort extracts by near infrared spectroscopy, high-performance liquid chromatography and capillary electrophoresis. Anal Chem Acta 2006;580:223–30.
[41] Zhang C, Shen Y, Chen J, Xiao P, Bao J. Nondestructive prediction of total phenolics, flavonoid contents, and antioxidant capacity of rice grain using near-infrared spectroscopy. J Agric Food Chem 2008;56:8268–72.
[42] Yu W-Y, Sun S-Q, Zhou Q, Leung H-W. Fourier transform mid-infrared (MIR) and near-infrared (NIR) spectroscopy for rapid quality assessment of Chinese medicine preparation Honghua Oil. J Pharm Biomed Anal 2008;46:498–504.
[43] Lu B, Chen J, Huang W, Wu D, Xu W, Xie Q, et al. Determination of flavonoids and phenolic acids in the extract of bamboo leaves using near infrared spectroscopy and multivariate calibration. Afr J Biotechnol 2011;10:8448–845.
[44] Chen Q, Jiang P, Zhao J. Measurement of total flavone content in snow lotus (Saussurea involucrata) using near infrared spectroscopy combined with interval PLS and genetic algorithm. Spectrochim Acta A Mol Biomol Spectrosc 2010;76:50–5.
[45] González-Martín I, Hernández-Hierro JM, Bustamante-Rangel M, Barros-Ferreiro N. Near-infrared spectroscopy (NIRS) reflectance technology for the determination of tocopherols in alfafla. Anal Bioanal Chem 2006;386:1553–8.
[46] Lau C-C, Chan C-O, Chau F-T, Mok DK-W. Rapid analysis of Radix puerariae by near-infrared spectroscopy. J Chromatogr A 2009;1216:2130–5.
[47] Zhou X, Xiang B, Wang Z, Zhang M. Determination of quercetin in extracts of ginkgo biloba leaves by Near-infrared reflectance spectroscopy based on interval partial Least-Squares (iPLS) model. Anal Lett 2007;40:3383–91.
[48] Wilson N, Heinrich M. The use of near infrared spectroscopy to discriminate between THC-rich and hemp forms of Cannabis. Planta Med 2006;72:260.
[49] Liu Y-M, Sheu S-J. Determination of the six major flavonoids in Scutellariae Radix by micellar electrokinetic capillary electrophoresis. Anal Chim Acta 1994;288:221–6.
[50] Horvath CR, Martins PA, Saxena PK. Identification and quantification of eight flavones in root and shoot tissues of the medicinal plant Huang-qin (Scutellaria baicalensis Georgii) using high-performance liquid chromatography with diode array and mass spectrometric detection. J Chromatogr A 2005;1062:199–207.
[51] Motoo Y, Sawabu N. Antitumor effects of saikosaponins, baicalin and baicalein on human hepatoma cell lines. Cancer Lett 1994;86:91–5.
[52] Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents 2005;26:343–56.
[53] Huang Q, Pan R, Wei J, Wu Y, Zhang L. Determination of baicalin and total flavonoids in Radix scutellariae by near infrared diffuse reflectance spectroscopy. Spectrosc Spectr Anal 2009;29:2425.
[54] Yang N, Ren G. Application of near-infrared reflectance spectroscopy to the evaluation of rutin and D-chiro-Inositol contents in tartary buckwheat. J Agric Food Chem 2008;56:761–4.
[55] Schönbiglsher S, Bittner LKH, Pallua JD, Popp M, Abel G, Bonn GK, et al. Simultaneous quantification of verbena in and baicalein in human hepatoma cell lines. Cancer Lett 1994;86:91–5.
[56] Yu C-Y. Quantification of phenolic compound in Magnolia officinalis herb by near infrared reflectance spectroscopy. J Zhejiang For Coll 2007;05.
[57] Schönbiglsher S, Bittner LKH, Pallua JD, Popp M, Abel G, Bonn GK, et al. Simultaneous quantification of verbena in and baicalein in human hepatoma cell lines. Cancer Lett 1994;86:91–5.
[57] Font R, Del Rio-Clestino M, Rosa E, Aires A, De Haro-Bailon A. Glucosinolate assessment in Brassica oleracea leaves by near-infrared spectroscopy. J Agric Sci 2005;143:65–73.

[58] Bala M, Singh M. Non destructive estimation of total phenol and crude fiber content in intact seeds of rapeseed—mustard using FTNIR. Ind Crops Prod 2013;42:357–62.

[59] Cordella C, Moussa I, Martel A-C, Sbirrazzuoli N, Lizzani-Cuvelier L. Recent developments in food characterization and adulteration detection: technique-oriented perspectives. J Agric Food Chem 2002;50:1751–64.

[60] He Y, Li X, Deng X. Discrimination of varieties of tea using near infrared spectroscopy by principal component analysis and BP model. J Food Eng 2007;79:1238–42.

[61] Huck-Pezzei VA, Bittner LK, Pallua JD, Sonderegger H, Abel G, Popp M, Bonn GK, Huck CW. A chromatographic and spectroscopic analytical platform for the characterization of St John’s wort extract adulterations. Anal Methods 2013;6:616–28.

[62] Li W, Xing L, Fang L, Wang J, Qu H. Application of near infrared spectroscopy for rapid analysis of intermediates of Tanreqing injection. J Pharm Biomed Anal 2010;53:350–8.

[63] Zalacain A, Ordoudi SA, Díaz-Plaza EM, Carmona M, Blázquez I, Tsimidou MZ, et al. Near-infrared spectroscopy in saffron quality control: determination of chemical composition and geographical origin. J Agric Food Chem 2005;53:9337–41.

[64] Pallua JD, Pezzei C, Huck-Pezzei VA, Schonbichler SK, Bittner LK, Bonn G, et al. Advances of infrared spectroscopic imaging and mapping technologies of plant material. Curr Bioact Compd 2011;7:12.