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Detection of illegally added drugs in dietary supplements by near-infrared spectral imaging

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The application to detect illegally added drugs in dietary supplements by near-infrared spectral imaging was studied with the focus on nifedipine, diclofenac and metformin. The method is based on near-infrared spectral images correlation coefficient to detect illegally added drugs. The results comply 100% with HPLC methods test results with no false positive results.

Keywords: Near-infrared; spectral imaging; illegally added; dietary supplements.

1. Introduction

Spectral imaging technology is a combination of traditional optical imaging and spectroscopy which acquires physical image (spatial information) and spectra (chemical information) simultaneously. Spectral imaging can clearly visualize the distribution of different components in the material by combining the spectral characteristics and image display.[1,2] Spectral imaging was previously applied to remote sensing measurements, such as geography,[3] agriculture,[4] marine,[5] environmental[6] and military fields measurement[7] according to the spectral resolving power which is divided into Multispectral Imaging and Hyperspectral Imaging. In recent years, with the rise of process analytical technology, modern chemometrics methods have been introduced into spectral image data recognition and processing. Spectral imaging instrument has gradually come from space into laboratory and field, to become a platform for analysis and detection technology which can be achieved from a single molecule scale[8,9] into the cell scale[10–12] measurements.

Spectral imaging technology in accordance with different spectroscopy can be divided into UV-visible spectral imaging, fluorescence spectral imaging, near infrared spectral imaging, mid-infrared spectral imaging, Raman spectral imaging and NMR imaging.[13,14] Traditional infrared spectroscopy obtained the average spectrum of a single point or small area, which is the average composition of the sample or the characteristics, ideally suited for the analysis of homogeneous material. Macro-infrared spectral imaging technology is complementary to traditional IR techniques, which can achieve the spatial and concentration

distribution of different components in the non-uniformly mixed sample. Because the samples used are mostly solid with simple preparation, no chemical and biological contaminations are produced. Spectral imaging technology is fast, sensitive and accurate, has obtained attention and been applied in biology,[15–19] medicine,[20] pharmacy,[21] agronomy[22] and food[23] and other areas. This paper discusses the application of near-infrared spectral imaging technology in the detection of illegally added drugs in dietary supplements.

2. Methods and Materials

2.1. *Instrument and materials*

The Perkin-Elmer Spotlight 400 FT-NIR spectral imaging system (Perkin-Elmer Inc., Waltham, Massachusetts, USA) with 1×16 MCT Line Array detectors was used to acquire near-infrared spectral image. Spectrum IMAGE spectral imaging workstation was applied to process the images.

All the dietary supplements samples used in the experiment were bought from the market. There are 38 kinds of positive samples containing nifedipine, 13 kinds of positive samples containing diclofenac sodium and 35 kinds of positive samples containing metformin. The concentration of illegally added drugs in these samples was determined by HPLC in advance. Nifedipine, diclofenac sodium and metformin reference standards are provided by National Institutes for Food and Drug Control. Betacyclodextrin with batch no. 20110601 is from Hubei Zhongjia Pharmaceutical Co., Ltd.

2.2. *Collection of NIR spectral image*

For solid reference standards, place the powdered reference sample on a clean glass slide, with another piece of clean glass slide gently flatten the reference, and then place the slide with reference on the microscope stage of imaging system. Select the near-infrared light source, reflection mode, spectral range 4000–7800 cm^{-1}, resolution of 8 cm^{-1}, use 99% Spectralon as background, the spatial resolution of 50 μm, image size 1000×1000 μm, to conduct near-infrared spectral imaging. Then take appropriate amount of sample, place on a clean glass slide, in accordance with the methods and parameters above for near-infrared spectral imaging.

2.3. *Processing of NIR spectral image*

We used the near-infrared spectra of reference standards as reference to calculate the sample near-infrared spectral images full spectral range correlation coefficient by spectral image processing software Spectrum-IMAGE. Spectra and spectral image processing methods include noise reduction, atmospheric correction, maximum and minimum normalization (min = 0, max = 1.5).

2.4. *Detection limits*

Mix adequate amount of nifedipine reference standard with betacyclodextrin at a mass ratio of 1:1000, 1:500, 1:200, 1:100 and 1:10 to investigate the detection limits of the method. Then mix diclofenac sodium reference standard with betacyclodextrin and metformin reference standard with betacyclodextrin in the same way to investigate the detection limits of the method.

3. Results

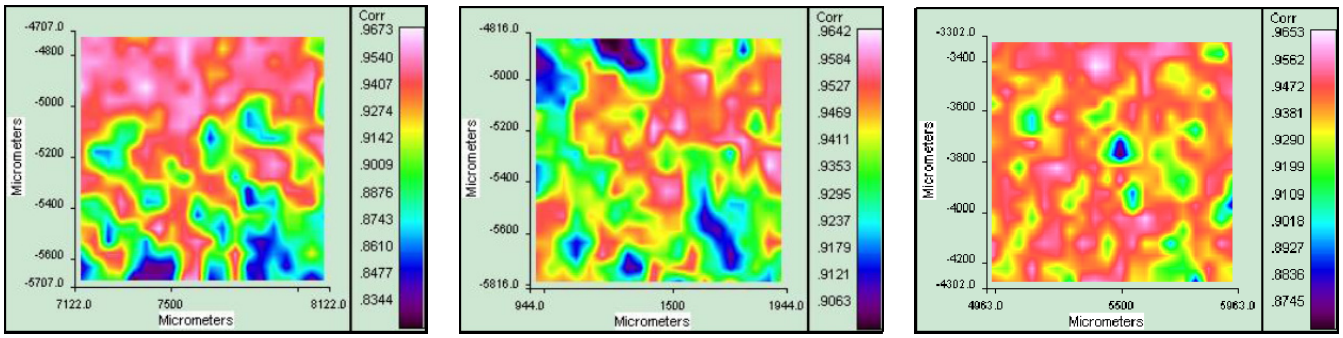
3.1. *Detection limits*

As shown in Fig. 1 and Table 1, nifedipine correlation coefficients in the mass fraction of 1% reach 0.95. In summary, the method can detect nifedipine in the mass fraction of more than 1% in betacyclodextrin. Diclofenac sodium correlation coefficients in the mass fraction of 100 reach 0.95. In summary, the method can detect diclofenac sodium in the mass fraction of more than 100th in betacyclodextrin. Metformin correlation coefficients in the mass fraction of 1% reach 0.95. In summary, the method can detect metformin in the mass fraction of more than 1% in betacyclodextrin.

3.2. *Detection of nifedipine*

Both positive samples with nifedipine and one negative sample without nifedipine were included (Table 2). The nifedipine concentration of these samples was determined by HPLC beforehand.

As shown in Fig. 2 and Table 3, of all the 39 kinds of dietary supplements samples near-infrared spectral images correlation coefficients with nifedipine reference, there are 28 kinds at 0.950 or more, there are six kinds in between 0.900 and 0.950, there are five kinds at 0.900 or less, in which one kind is No. 39 the negative sample, for the other four kinds of samples,



(a) (b) (c)

Fig. 1. Detection limits of the method used in nifedipine (a), diclofenac sodium (b) and metformin (c) mixed with betacyclodextrin.

Table 1. Detection limits of the method used in nifedipine, diclofenac sodium and metformin mixed with betacyclodextrin.

Mass ratio	1:1000	1:500	1:200	1:100	1:10
Nifedipine correlation coefficient	0.384	0.413	0.582	0.961	0.972
Diclofenac sodium correlation coefficient	0.412	0.561	0.952	0.977	0.986
Metformin correlation coefficient	0.512	0.661	0.732	0.962	0.979

Table 2. Positive samples with nifedipine and negative samples without nifedipine.

No.	1	2	3	4	5	6	7	8	9	10	11	12	13
Amount in one capsule/mg	1.5	4.4	2.4	2.0	1.8	1.7	4.5	2.8	2.4	1.8	1.0	2.6	1.7
No.	14	15	16	17	18	19	20	21	22	23	24	25	26
Amount in one capsule/mg	3.0	3.5	1.0	3.1	1.6	2.5	4.0	1.9	1.4	5.0	3.8	1.6	6.1
No.	27	28	29	30	31	32	33	34	35	36	37	38	39
Amount in one capsule/mg	1.1	4.1	4.2	2.4	1.4	3.4	2.3	3.3	1.3	5.6	0.5	3.6	0

the near-infrared spectral images correlation coefficient is small due to the lower concentration. This method results in line with the known rate of 100%.

3.3. *Detection of diclofenac sodium*

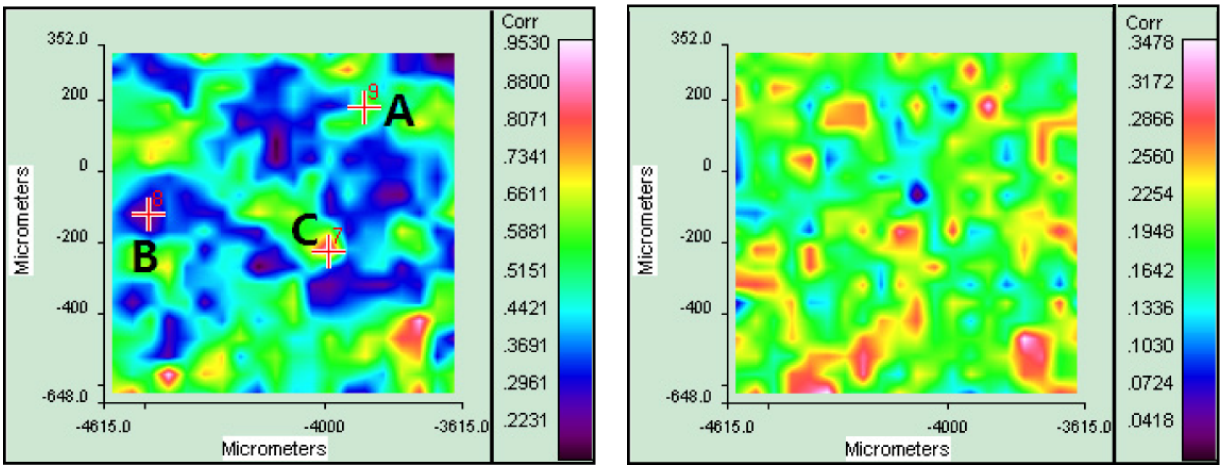
Both positive samples with diclofenac sodium and one negative sample without diclofenac sodium were included (Table 4). The diclofenac sodium concentration of these samples was determined by HPLC beforehand.

As showed in Fig. 3 and Table 5, all 13 kinds of dietary supplements positive samples near-infrared spectral images correlation coefficients with Diclofenac sodium reference are more than 0.950, the No. 14 sample is the negative sample, and the correlation coefficient is small. This method results in line with the known rate of 100%.

3.4. *Detection of metformin*

Both positive samples with metformin and one negative sample without metformin were included (Table 6). The metformin concentration of these samples was determined by HPLC beforehand.

As shown in Fig. 4 and Table 7, all 35 kinds of dietary supplements positive samples near-infrared spectral images correlation coefficients with metformin reference are 0.950 or more, the No. 36 sample was the negative sample, and the correlation coefficient is small. This method results in line with the known rate of 100%.



(a) (b)

Fig. 2. Nifedipine near-infrared spectral images correlation coefficient of sample No. 23 (a) and sample No. 39 (b), with correlation coefficient $A = 0.902$, $B = 0.281$, $C = 0.542$.

Table 3. Nifedipine near-infrared spectral images correlation coefficient.

No.	1	2	3	4	5	6	7	8	9	10	11
Correlation coefficient	0.902	0.976	0.967	0.954	0.966	0.968	0.962	0.975	0.951	0.957	0.759
No.	12	13	14	15	16	17	18	19	20	21	22
Correlation coefficient	0.976	0.966	0.979	0.980	0.869	0.965	0.936	0.963	0.970	0.970	0.913
No.	23	24	25	26	27	28	29	30	31	32	33
Correlation coefficient	0.952	0.970	0.929	0.976	0.858	0.975	0.969	0.964	0.940	0.960	0.976
No.	34	35	36	37	38	39					
Correlation coefficient	0.955	0.938	0.975	0.767	0.965	0.165					

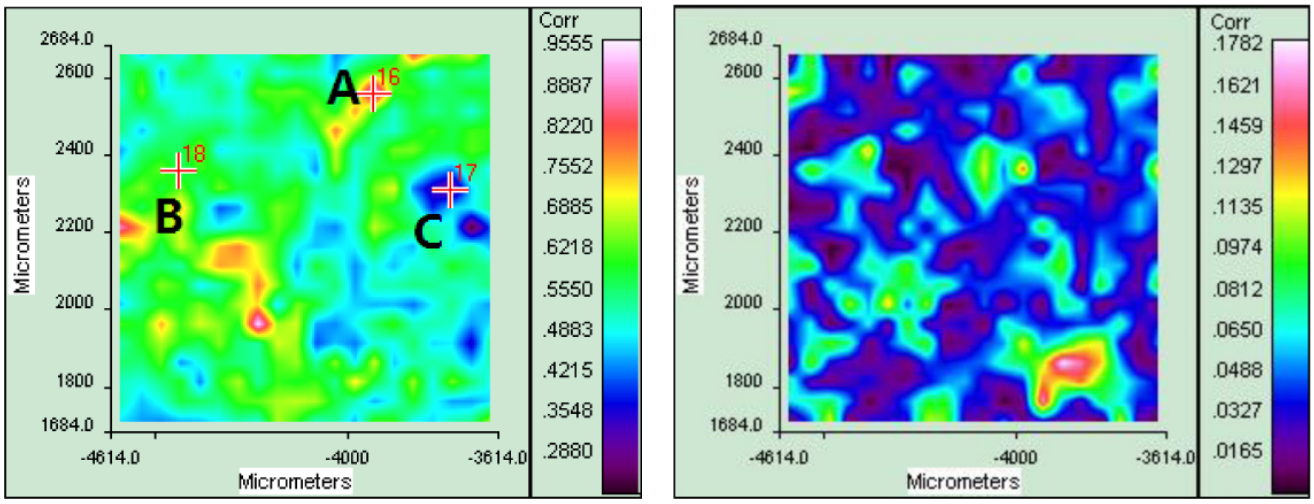
Table 4. Positive samples with diclofenac sodium and negative samples without diclofenac sodium.

No.	1	2	3	4	5	6	7
Amount in one capsule/mg	5.4	18.9	12.0	17.6	5.9	9.2	9.5
No.	8	9	10	11	12	13	14
Amount in one capsule/mg	10.9	9.5	14.3	9.2	8.2	14.0	0

4. Discussion

In previous studies, HPLC[24] and HPLC-MS[25] had been applied to the detection of illegally added drugs in the dietary supplement. HPLC method has the detection limit of 1–80 ng and HPLC-MS method has the limit detection of 1–5 ng.

The method in this paper is based on near-infrared spectral images correlation coefficient to detect illegally added drugs nifedipine, diclofenac and metformin in dietary supplements. The detection limit due to the characteristics of near-infrared spectroscopy cannot reach the level of HPLC and HPLC-MS methods. However, it takes hours even days to carry out HPLC and HPLC-MS methods while the near-infrared spectral imaging only takes minutes. The agility is the most obvious advantage of spectral imaging which can provide initial



(a) (b)

Fig. 3. Diclofenac sodium near-infrared spectral images correlation coefficient of sample No. 6 (a) and sample No. 14 (b), with correlation coefficient $A = 0.952$, $B = 0.576$, $C = 0.319$.

Table 5. Diclofenac sodium near-infrared spectral images correlation coefficient.

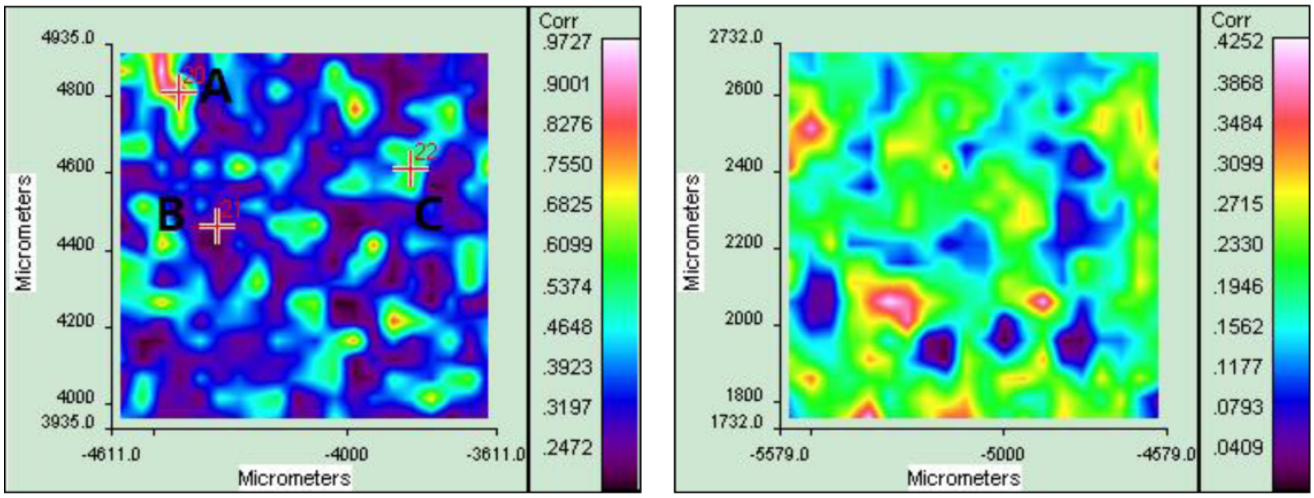
No.	1	2	3	4	5	6	7
Correlation coefficient	0.958	0.989	0.958	0.964	0.974	0.952	0.953
No.	8	9	10	11	12	13	14
Correlation coefficient	0.958	0.989	0.958	0.964	0.974	0.952	0.143

Table 6. Positive samples with metformin and negative samples without metformin.

No.	1	2	3	4	5	6	7	8	9	10	11	12
Amount in one capsule/mg	53.1	28.9	50.4	18.9	79.0	15.4	30.4	10.3	23.2	15.3	21.9	9.1
No.	13	14	15	16	17	18	19	20	21	22	23	24
Amount in one capsule/mg	34.3	27.2	15.3	17.4	24.1	20.4	16.3	27.3	22.7	34.4	9.0	44.2
No.	25	26	27	28	29	30	31	32	33	34	35	36
Amount in one capsule/mg	123.8	15.1	38.5	76.5	61.1	42.9	12.5	28.8	39.6	38.8	77.1	0

screening of illegally added drugs in dietary supplements before conducting more precise and time-consuming methods. The results of near-infrared spectral imaging comply 100% with HPLC methods test results with no false positive results.

This method used in this experiment only chooses three kinds of illegally added drugs. Future detection has the potential of covering expanded range of other illegal substances added to the dietary supplements. This is an application of line array detector based on near-infrared spectral imaging in drug quality control, that provides a new solution to combat counterfeit drugs and illegally added drugs in dietary supplements.



(a) (b)

Fig. 4. Metformin near-infrared spectral images correlation coefficient of sample No. 1 (a) and sample No. 36 (b), with correlation coefficient $A = 0.973$, $B = 0.268$, $C = 0.604$.

Table 7. Metformin near-infrared spectral images correlation coefficient.

No.	1	2	3	4	5	6	7	8	9
Correlation coefficient	0.973	0.951	0.991	0.952	0.988	0.964	0.966	0.987	0.96
No.	10	11	12	13	14	15	16	17	18
Correlation coefficient	0.967	0.984	0.993	0.956	0.998	0.985	0.995	0.99	0.998
No.	19	20	21	22	23	24	25	26	27
Correlation coefficient	0.985	0.956	0.954	0.978	0.969	0.991	0.973	0.972	0.971
No.	28	29	30	31	32	33	34	35	36
Correlation coefficient	0.975	0.985	0.972	0.957	0.971	0.994	0.994	0.956	0.217

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