Simultaneous Determination of Synthetic Colorants in Pickles and Sauerkrauts by HPLC-MS

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Abstract:The Objective of this paper is to establish a method for determination of six synthetic colorants (sunset yellow, tartrazine, amaranth, carmine, allura red, brilliant blue)in pickles and sauerkrauts. The samples were adsorbed by polyamide then desorbed by aqueous alkali. After purified and extracted, the samples were detected by reverse phase liquid chromatography using mobile phase of methanol and acetic ammonium solution (the concentration is 0.02mol/L), mixture under gradient elution, confirmed and quantified by mass spectrum in the selected reaction monitoring (SRM) mode via positive electrospray ionization (ESI+).The results show that the method of sample testing had strong qualitative and quantitative ability. The curve of peak area with concentration was from 0.1 to 50 mg/L with correlation coefficients from 0.9990 to 0.9999. The detection limit of these six synthetic colorants was between 0.4 and 13 µg/kg. The recovery of the method was from 89.0% to 101.2%, and the relative standard deviation (RSD) was between 0.5% and 1.6%. The method developed is sensitive, simple, accurate and can be used to qualify and quantify this six synthetic colorants in pickles and sauerkrauts.

Introduction

The source of food colorants can be divided into natural pigments and synthetic colorants in two categories, including synthetic colorant, which has been used widely with its bright colors and low price. However, synthetic colorants were made from aniline in coal tar as raw material, which has been demonstrated to cause negative effects on human health in case of overdose. Therefore, all countries in the world have a strict control on the scope and quantity of use. At present, the main method for detection of synthetic colorants were high performance liquid chromatography[1,2,3,4], thin layer chromatography[5,6], oscillopolarography[7]. Most of these methods are low sensitivity, interference and so on in determination of colorants. Especially the composition in pickled vegetables is complex, many interference factors exist, it is easier to make misjudgment and omission of judgment. Some researcher made some analysis methods together[8,9]. So the method is established in this paper to determine six synthetic colorants in pickles and sauerkrauts simultaneously. It is used with High performance liquid chromatography-mass spectrometry and mass spectrometry at the same time, which can solve the problems in the above methods.

Materials and Experimental Methods

Chemicals and Apparatus Methanol and ammonium acetate were purchased from Tedia(American International Chemical, Inc. California, USA).their purity were Chromatographic Pure . Sunset yellow, tartrazine, amaranth, carmine, allura red, brilliant blue standards were purchased from the national standard material research center. Methanoic acid, ammonia water, ethanol were purchased from GuangHua Company(Guangzhou, China). All of these reagents were analytically pure. Polyamide was purchased from GuangHua Company (Guangzhou, China). The size of powder: 100-200 mesh. Water was purified using a Milli-Q Ultra-pure water purification system (Millipore, Bedford, MA, USA).

Triple quadrupole mass spectrometer, America's Quantum Access quaternary gradient pump, Accela, automatic sampler, and Xcalibur workstation were purchased from Thermo Fisher Company (USA). Ultra high performance liquid chromatograph (HPLC)and mass spectrometer (MS) were purchased from UPLC Waters Company (USA),Waters Symmetry C18 chromatographic column (50mm×2.1mm，1.9μm) was purchased from Waters Company (USA). 0.22μm nylon filter was got from Pall Corporation(USA).All other reagents were analytically pure.

Experiment Condition of HPLC and MS Thermo Hypersil GOLD(50mm×2.1mm，1.9μm) column were used. The Column temperature was maintained at 30℃. The injected sample volume was 10μl. The flow-rate used was 200μL/min.. The mobile phase was a mixture of methanol and 0.02M ammonium acetate, and a gradient elution method was used. The optimized chromatographic condition was: 0~2min, the concentration of methanol is 15%; 2~3min, linear variation of methanol is 98%，keeping time was 7min, 10～11min, linear variation of methanol is 15%，keeping time was 4min. Then return to initial conditions; a 10 min post-run time was used after each analysis. The column was conditioned prior to use by washing with Methanol and deionizes water successively.

Table 1 Choice reaction monitoring conditions of six kinds of colorants

Item	Parent ion(m/z)	Daughter ion(m/z)	Collision energy(V)	Lens compensation(V)
sunset yellow	409	173	20	69
		136	22	
tartrazine	469	451	23	72
		200	20	
amaranth	539	348	27	106
		223	38	
carmine	539	223	25	142
		158	25	
allura red	453	217	26	97
		202	31	
brilliant blue	749	171	48	154
		306	47	

This HPLC system was connected to a mass spectrometer (HPLC Waters Company ,USA), equipped with an electrospray interface operating in negative ion, using the following operation parameters: capillary voltage 3500 V, electro spray ionization(ESI), in positive mode, selected reaction monitoring (SRM), resolution ratio: Q1=0.7M，Q3=0.7M, Sheath gas N_2, pressure 39 au, Aux gas N_2, pressure 10 au. Heat the temperature of the capillary: 350℃, Collision gas pressure, 1.5m Torr, sweep length(Scan Width m/z) 0.100, scanning time is 0.200s. Other conditions are shown in the Table 1.Choice ion current chromatograms of 6 kinds of colorants are shown in Fig.1.

Experimental Method Stirring pickles and sauerkrauts into pieces in a blender, about 5.00g (accurately weighed to 0.01g) of samples were weighed into a 30ml neck flask and dissolved with water, adjust pH to 6 by citric acid.1.00g (accurately weighed to 0.01g) of polyamide powder and a little water were mixed together uniformity then added to the solution of samples, ultrasound extracted for 15mins, suction filtration by G3 sintered funnel, washed 3~5 times through water, then washed 3~5 times by mixture of methanol and methanoic acid, then washed until neutral by water, desorbed 3~5 times by mixture of alcohol and ammonia water, 5ml each time, collect desorbing liquid, added acetic acid to neutral , vapored to almost dry, add water to solve. Finally, the neck flask was constant volume to 50ml with water. After being shaken and centrifuged, colorants were collected through a filter with pore size of 0.22μm before injection into the HPLC system.
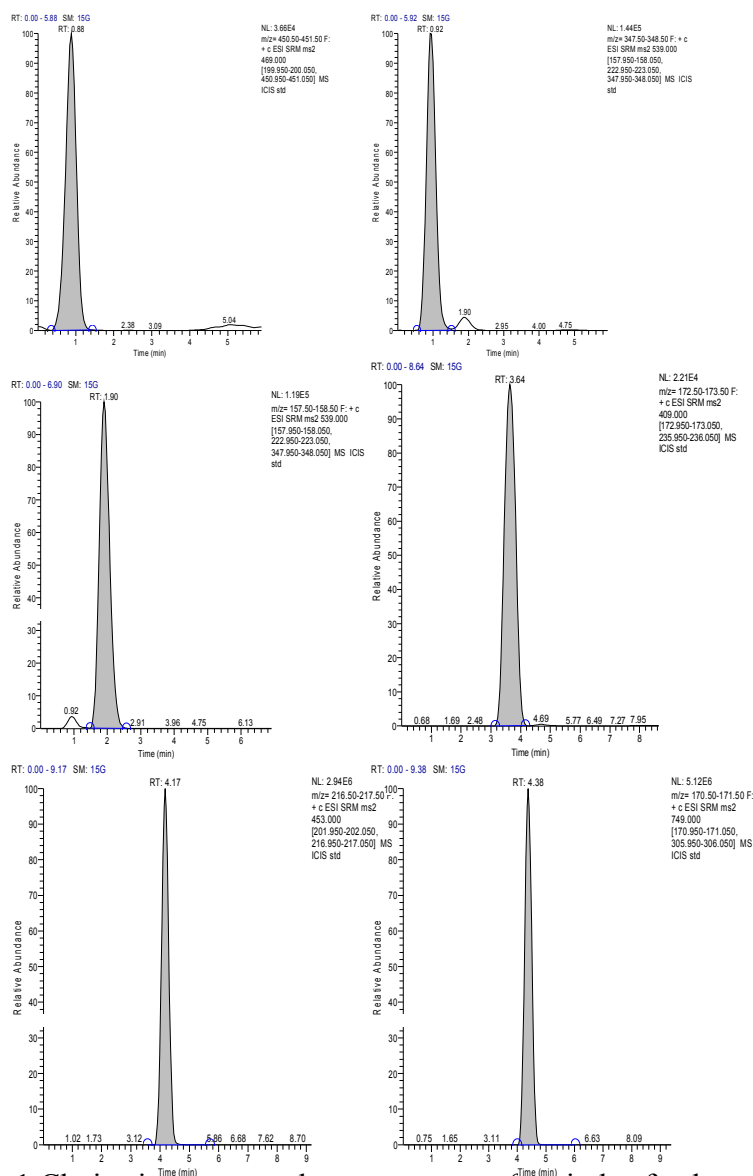
Fig. 1 Choice ion current chromatograms of 6 kinds of colorants

Result and Discussion

Choice Of Chromatographic Condition Using methanol and ammonium acetate(0.02M) as mobile phase, then gradient eluted and isocratic elution individually, the results show that not only acquired satisfied peak and degree of separation ,but also reduced the time of analysis and the noise of instrument.

    Calibration Curve Recovery Rate and Precision When the concentration between 0.1 and 50mg/L, The linear relation between peak area Y concentrate X of six synthetic colorants are good. The calibration curve of these synthetic colorants were shows in table 2.The results shows that the linearity of the analytical

Table 2 The equation of linear regression and correlation coefficient of the six synthetic colorants

Components name	regression equation	R
Tartrazine	Y=1599.1X+221	0.9990
Amaranth	Y=5879.8X+106	0.9995
Carmine	Y=5674.7X+331	0.9993
Sunset yellow	Y=1273.2X+138	0.9991
Allura red	Y=8162.9X+445	0.9996
Brilliant blue	Y=14128.9X+507	0.9999

response within the studied range of two orders of magnitude is excellent, with correlation coefficients higher than 0.9990 in all case.

Add 5.00mg (accurately weighed to 0.01g) of these synthetic colorants into a blank sample. After disposing, parallel test were done and calculate recovery rate , recovery and precision are showed in Table 3.All results showed that recovery rate was from 89.0% to 101.2%. The relative standard deviation (RSD) was between 0.5% and 1.6%.

Table3 Recovery rate and precision for the 6 kinds of synthetic colorants

Item	Sunset Yellow	Tartrazine	Amaranth	Carmine	Allura red	Brilliant blue
Estimated value	4.63	4.78	4.94	4.45	4.66	5.06
Recovery（%）	92.6	95.6	98.8	89.0	93.2	101.2
RSD（%）	1.6	1.0	0.8	1.9	1.1	0.6

Samples Test To evaluate the effectiveness of the proposed method, it was applied to the analysis of a total of 18 samples of pickles and sauerkrauts in market randomly. Fortunately, in most cases, most synthetic colorants were not found in the samples. Unfortunately, the results indicate that four samples which added sunset yellow is illegally. Testing results of samples were shown in Table 4.

Table 4 Testing results of all samples（the concentration unit is g/kg）

Sample	sunset yellow	tartrazine	amaranth	carmine	allura red	brilliant blue
Sample1	0.002	ND	0.004	ND	ND	ND
Sample2	ND	ND	0.008	ND	ND	ND
Sample3	ND	ND	ND	ND	ND	ND
Sample4	0.003	ND	0.003	0.006	ND	ND
Sample5	0.003	ND	0.003	ND	ND	ND
Sample6	ND	ND	ND	ND	ND	ND
Sample7	ND	ND	0.008	ND	ND	ND
Sample8	ND	ND	ND	ND	ND	ND
Sample9	ND	ND	ND	ND	ND	ND
Sample10	0.004	ND	0.004	0.008	ND	ND
Sample11	0.003	ND	0.007	ND	ND	ND
Sample12	ND	ND	0.011	ND	ND	ND
Sample13	ND	ND	ND	0.007	ND	ND
Sample14	0.003	ND	ND	ND	ND	ND
Sample15	0.004	ND	ND	ND	ND	ND
Sample16	ND	ND	ND	ND	ND	ND
Sample17	0.005	ND	0.003	0.008	ND	ND
Sample18	ND	ND	ND	ND	ND	ND

ND: it is not detected in sample.

Conclusion

This paper established a method of a high-performance liquid chromatography-mass spectrometry/mass spectrometry determination six synthetic colorants (lemon yellow, amaranth carmine, sunset yellow fcf, allura Red AC, brilliant blue) in pickles and sauerkrauts. The method of sample testing had strong qualitative and quantitative ability, fully applied in determination of samples. This is a real example that illustrates the useful determination for six synthetic colorants with the HPLC/MS simultaneously. The method developed is sensitive, simple, accurate and suitable

for the determination of six synthetic colorants in pickles and sauerkrauts simultaneously. It provided practical technical means to monitor synthetic colorants in pickles and sauerkrauts. It is important significance in the use of food additives. The method is rapid and has been shown to be reliable.

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