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Short communication

Determination of transition metal ions in tobacco as their 2-(2-quinolinylazo)-5-dimethylaminophenol derivatives using reversed-phase liquid chromatography with UV–VIS detection

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Abstract

This paper reports the utilization of solid-phase extraction and the reversed-phase high-performance liquid chromatography for the determination of six important transition metal ions: iron, cobalt, nickel, copper, zinc and manganese in tobacco with 2-(2-quinolinylazo)-5-dimethylaminophenol (QADMAP) as chelating reagent. Iron, cobalt, nickel, copper, zinc and manganese ions react with QADMAP to form colored chelates in the medium of acetic acid–sodium acetate buffer solution (pH 4.0). These chelates can be enriched by solid-phase extraction with Waters Sep-Pak-C₁₈ cartridge, and eluted the retained chelates from cartridge with tetrahydrofuran. The chelates were separated on a Waters Nova-Pak-C₁₈ column (150×3.9 mm, 5 μm) by gradient elution with methanol (containing 0.5% of acetic acid) and 0.05 mol/l pH 4.0 acetic acid–sodium acetate buffer solution as mobile phase at a flow-rate of 0.5 ml/min. The detection limits of iron, cobalt, nickel, copper, zinc and manganese are 10, 12, 8, 13, 17 and 22 ng/l, respectively. This method had been applied to the determination of iron, cobalt, nickel, copper, zinc and manganese in tobacco with good results.

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1. Introduction

The simultaneous determination of metal ions by RP-HPLC techniques as they chelate with organic reagents have been receiving increased attention in recent years [1–6]. Many azo dyes, such as pyridylazo reagents, thiazolylazo reagents, benzothiazolylazo reagents and similar have been widely used as chelating reagents for the simultaneous determination of metal ion by RP-HPLC [7–18], but

the 2-quinolinylazo derivatives have received little attention. In previous work, some 2-quinolinylazo reagents have been synthesized and applied to the spectrophotometric determination of metal ions [19–26]. This type of reagent has a higher sensitivity than pyridylazo reagents, thiazolylazo reagents and benzothiazolylazo reagents because of its larger conjugated system.

Iron, cobalt, nickel, copper, zinc and manganese are the most important transition metal ions in tobacco. The simultaneous determination of iron, cobalt, nickel, copper, zinc and manganese is very important for tobacco quality control [27]. In this work, in order to select a more sensitive azo dye for

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the simultaneous determination of iron, cobalt, nickel, copper, zinc and manganese, a new chelating reagent 2-(2-quinolinylazo)-5-dimethylaminophenol (QADMAP) was synthesized and its chelating reaction with iron, cobalt, nickel, copper, zinc and manganese was studied. A simple, sensitive and selective method for the simultaneous determination of iron, cobalt, nickel, copper, zinc and manganese in tobacco was developed, based on the solid-phase extraction of the metal chelates by Waters Sep-Pak-C₁₈ cartridge and the RP-HPLC separation.

2. Experimental procedures

2.1. Synthesis of QADMAP

The route for the synthesis of QADMAP is shown in Fig. 1, and the QADMAP was synthesized by our laboratory in the following procedure: 2-aminoquinoline (7.2 g) was dissolved in a 500-ml anhydrous ethanol. Sodamide (2.0 g) was added. Refluxed for 5 h in boiling water bath, then isoamyl nitrite (7.4 ml) was added. Refluxed for 30 min in boiling water bath. The solution was cooled and placed 1 night under 0 °C. Diazo salt was obtained by filtering this solution with a productivity of 95%. The diazo salt was dissolved in 200 ml anhydrous ethanol, and *m*-dimethylaminophenol (5.2 g) was added, ventilated the carbon dioxide into the solution with stirring until the pH reached about 8.0. The solution was placed for 2 days. Then the solution was diluted with 400 ml water and extracted with chloroform. The chloroform was evaporated and the residue was re-crystallized with 20% ethanol. QADMAP was obtained with a yield of 43%. The structure of QADMAP was verified by elemental analysis, IR, ¹HNMR, and MS. Elemental analysis: C₁₇H₁₆N₄O found (calculate) C 69.64 (69.85), N

18.96 (19.17), H 5.76 (5.52). IR (KBr) (cm⁻¹): 3610 (ν_{O-H}); 1050 (ν_{C-O}); 1600, 1565, 1520, 1430 (ν_{C=C}, -N=N-); 1375, 1326 (ν_{C-N}); 2855 (ν_{C-H}); 1465 (δ_{C-H}); 3070, 3016 (σ_{Ar-H}); 1175, 1120, 865, 775, 730 (δ_{Ar-H}). ¹HNMR (solvent: [²H₆]acetone) (δ ppm): 2.68 (s 6H, N-CH₃), 2.27 (s 1H, -OH); 6.86~7.85 (m 9H, Ar-H). MS: 292 (M⁺).

2.2. Chemicals and apparatus

The HPLC system consisted of a Waters 2690 Alliance separation model and a 996 photodiode array detector (Waters, Milford, MA, USA). The pH values were determined with a Beckman Φ-200 pH meter. The separation column used was Waters Nova-Pak-C₁₈ column (150×3.9 mm, 5 μm). A Waters solid-phase extraction (SPE) device, which can prepare 20 samples simultaneously and a Waters Sep-Pak-C₁₈ solid-phase extraction cartridge (1 ml/30 mg, 30 μm) was also used.

All of the solutions were prepared with ultra-pure water obtained by a Milli-Q50 SP Reagent Water System (Millipore, Bedford, MA, USA). QADMAP was dissolved with tetrahydrofuran (THF) to make a 1.0×10⁻⁴ mol/l of solution. Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and Mn(II) standard solution: 1.0 mg/ml (obtained from Chinese Standards Material Center), a working solution of 0.5 μg/ml was prepared by diluting this standard solution. HPLC grade methanol and THF (Fisher). Acetic acid-sodium acetate buffer solution: 0.5 mol/l, pH 4.0. Mobile phase A: methanol (containing 0.5% of acetic acid). Mobile phase B: 0.05 mol/l of acetic acid-sodium acetate buffer solution (pH 4.0). All other reagents used were of analytical-reagent grade. The glass and PTFE wares used were soaked in 5% of nitric acid for a period of time, and then thoroughly washed with pure water.

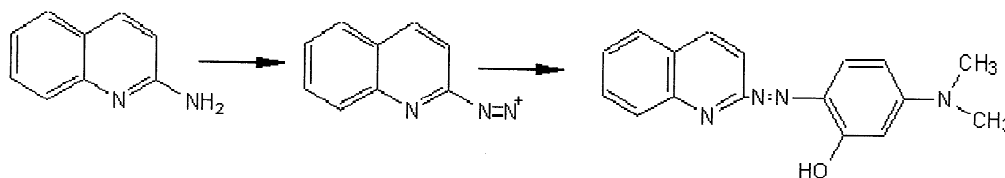


Fig. 1. The route for the synthesis of QADMAP.

2.3. General procedure

An appropriate volume (not more than 17 ml) of digested sample or standard solution was transferred in a 25-ml volumetric flask, to which 5.0 ml of a 1.0×10^{-4} mol/l of QADMAP THF solution and 3.0 ml of a 0.5 mol/l acetic acid–sodium acetate buffer solution (pH 4.0) was added and diluted to the volume with water. After 10 min the solution was passed through the Waters Sep-Pak C₁₈ cartridge at a flow-rate of 10 ml/min. The chelates were retained on the cartridge. After the enrichment finished, the retained chelates were eluted from the cartridge with 1.0 ml of THF at a flow-rate of 5 ml/min in an opposite direction. The THF solution filtered with 0.45 μm of filters and volatilized to 0.6 ml, then diluted to the volume of 1.0 ml with water, injected with 20 μl for HPLC analysis. A tridimensional chromatogram was recorded from 450 to 650 nm with photodiode array detector and the chromatogram of 550 nm was shown in Fig. 2. The composition of mobile phase during gradient elution is: 0 min (60% A and 40% B), 15 min (100% A and 0% B) in linear ramp. Each metal–QADMAP chelate was detected at its maximum absorption wavelength. A reagent blank test prepared in a similar way without sample addition was also carried out.

2.4. Application to tobacco sample

A total of 0.25 g of tobacco samples were weighted accurately into the PTFE high-pressure microwave acid-digestion bomb (Fei Yue, Analytical Instrument Factory, Shanghai, China) and 2.5 ml of concentrated nitric acid and 2.5 ml of 30% hydrogen peroxide were added. The bombs were sealed tightly and then positioned in the carousel of the microwave oven (Model WL 5001, 1000 W, Fei Yue Analytical Instrument Factory). The system was operated at full power for 6.0 min, then the digest was evaporated to near dryness. The residue was dissolved with 5 ml of 5% hydrochloric acid and transferred into a 20-ml calibrated flask quantitatively and diluted to volume with water. The Fe, Co, Ni, Cu, Zn and Mn contents were analyzed by using a proper volume of solution according to general procedure, together with the results of a recovery test by adding 0.5 μg of Fe, Co, Ni, Cu, Zn and Mn in 0.25 g of tobacco sample. The

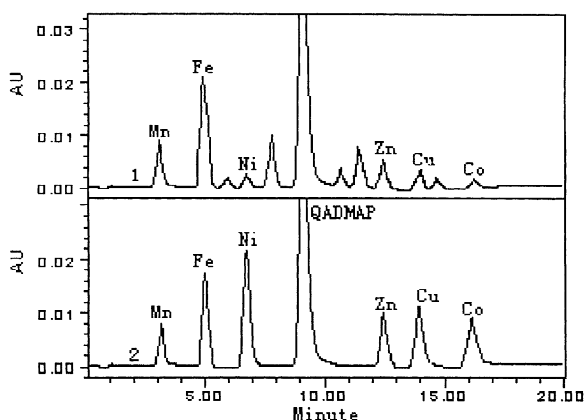


Fig. 2. Chromatogram of standard sample and tobacco sample: (1) tobacco sample, (2) standard sample. Injection volume 20 μl . The concentration of Fe, Co, Ni, Zn, Cu, Mn was 20 $\mu\text{g/l}$ in standard sample. Detection wavelength is 550 nm. Other conditions as in standard procedure.

results are shown in Table 1. A standard method using inductively coupled plasma (ICP) MS has also been used as a reference method. The results are shown in Table 2. The peak purities of Fe–QADMAP, Co–QADMAP, Ni–QADMAP, Cu–QADMAP, Zn–QADMAP and Mn–QADMAP chelates were identified by photodiode array detector to ensure there were no foreign peaks overlapping with these.

3. Result and discussion

3.1. Precolumn derivatization

In a weak acid medium of pH 2.5–6.0, Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and Mn(II) can form stable and colored chelates with QADMAP, so a 0.5-mol/l, pH 4.0, acetic acid–sodium acetate buffer solution was recommended to control pH. It was found that 1.0 ml of 1.0×10^{-4} mol/l QADMAP THF solution was sufficient to chelate 10.0 μg of Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and Mn(II) ions. However, in the tobacco samples, the foreign ions would react with QADMAP to consume reagents. More QADMAP was needed. The experiments showed that 5.0 ml of QADMAP solution was sufficient to chelate the ions in tobacco samples, and

Table 1
Determination results ($\mu\text{g/g}$) of the sample with this method

Components	Samples ($\mu\text{g/g}$)				RSD (%, $n=5$)	Recovery (% , $n=5$) added 0.5 μg metal ions
	Qujing (B_2F)	Yuxi (X_3F)	Dali (C_3F)	Zhaotong (C_1F)		
Fe	248	165	215	221	2.1~3.2	93~107
Co	2.05	1.26	1.86	1.52	2.3~3.6	94~106
Ni	4.36	2.23	2.74	3.23	1.8~3.0	92~106
Cu	9.47	5.47	8.18	6.82	2.3~3.5	94~107
Zn	15.8	11.6	15.4	12.5	2.4~3.6	91~106
Mn	72.5	48.3	56.6	58.3	1.7~2.9	93~106

so, 5.0 ml was recommended. The metal–QADMAP chelates have a poor solubility in aqueous solution. It is needed to add a proportion of organic solvent to improve their solubility. Several routine organic solvents were studied to enhance the solubility of chelates. The effects of the various organic solvents were in the following sequence: THF>acetone>acetonitrile>ethanol>methanol. THF was selected. The amount of THF addition within 4.5~7.0 ml have good effects for improving the solubility and do not affect the chelates enrichment, so a 5.0-ml of QADMAP THF solution was recommended.

3.2. Solid-phase extraction

Both the enrichment and the elution were carried out on a Waters SPE device, which can prepare 20 samples simultaneously. The flow-rate was set to 10 ml/min when enrichment and 5 ml/min when elution.

Some experiments were carried out in order to investigate the retention of metal–QADMAP chelate on the cartridge. It was found that the Fe–QADMAP, Co–QADMAP, Ni–QADMAP, Cu–QADMAP, Zn–QADMAP and Mn–QADMAP chelates could be retained on the cartridge quantitatively when they

pass the cartridge as an aqueous solution. The capacity of the cartridge for QADMAP was 20 mg and for its metal–QADMAP chelate was 18 mg in a 25-ml solution. In this experiment, the cartridge has adequate capacity to enrich the metal–QADMAP chelates and the excess QADMAP.

In order to choose a proper eluent for the retained QADMAP and its metal chelates, various organic solvents were studied. It was found that the THF, acetone, acetonitrile, ethanol and methanol could elute the QADMAP and its metal chelate from the cartridge quantitatively. The effect of the various eluents for the retained QADMAP and its metal chelate was in the following sequence: THF>acetone>acetonitrile>ethanol>methanol. THF was selected. The metal–QADMAP chelate has a good stability in a weak acid medium. Containing 0.3~0.7% of acetic acid in THF could increase the stability of the metal–QADMAP chelate during elution. THF (containing 0.5% acetic acid) was selected as eluent. Experiments showed that it was easier to elute the retained QADMAP and its metal chelate on cartridges in the reverse direction than in the forward direction, so it was necessary to upturn the cartridge when elution. A total of 1.0 ml of THF (containing 0.5% of acetic acid) was sufficient for

Table 2
Determination results ($\mu\text{g/g}$) of the sample with ICP-MS method

Components	Samples ($\mu\text{g/g}$)				RSD (%, $n=5$)	Recovery (% , $n=5$) added 0.5 μg metal ions
	Qujing (B_2F)	Yuxi (X_3F)	Dali (C_3F)	Zhaotong (C_1F)		
Fe	253	161	218	229	2.6~3.8	88~106
Co	2.11	1.22	1.78	1.43	2.5~3.9	89~108
Ni	4.31	2.16	2.81	3.12	2.3~3.7	89~104
Cu	9.41	5.55	8.26	6.73	2.7~3.6	92~108
Zn	15.1	12.4	16.2	11.8	2.8~3.8	91~107
Mn	71.6	48.8	56.1	58.8	2.7~3.9	89~108

elution of the QADMAP and its metal chelate from cartridge quantitatively at a flow-rate of 5 ml/min. The volume of 1.0 ml eluent was selected.

3.3. Chromatographic separation

The Fe–QADMAP, Co–QADMAP, Ni–QADMAP, Cu–QADMAP, Zn–QADMAP and Mn–QADMAP chelates have a good stability in weak acid medium. The pH of the mobile phase should be within 2.5–6.0 to avoid the chelates decomposing in the course of separation and get a good peak shape. Two weak acid solutions, mobile phase A: methanol (containing 0.5% of acetic acid) and mobile phase B: 0.05 mol/l acetic acid–sodium acetate buffer solution (pH 4.0) were recommended, and a Waters Nova-Pak-C₁₈ column (150×3.9 mm, 5 μm) was selected as analytical column in this experiment. The results showed that the gradient elution can get a better result than the isocratic elution. Gradient elution was selected. The proper composition of mobile phase in the course of gradient elution was selected as following: 0 min (60% A and 40% B), 15 min (100% A and 0% B) in linear ramp.

3.4. Spectrophotometric properties

From the tridimensional chromatogram recorded by photodiode array detector, the absorption spectrum of metal–QADMAP chelates was obtained. The maximum absorption wavelengths of Fe–QADMAP, Co–QADMAP, Ni–QADMAP, Cu–QADMAP, Zn–QADMAP and Mn–QADMAP were 560, 572, 555, 538, 545 and 525 nm. To get a maximum sensitivity, each metal–QADMAP chelate was monitored at its maximum absorption wavelength.

3.5. Calibration graphs

Under the optimum conditions, the regression equations of metal–QADMAP chelates were established based on the standard samples injected and their peak area. The limits of detection were calculated by the ratio of signal-to-noise ($S/N=3$). The results were shown in Table 3. The reproducibility of this method was also examined for 20 μg/l of Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and Mn(II). The relative standard deviations ($n=10$) are shown in Table 3.

3.6. Interference

Under the pre-column derivatization conditions, the foreign ions of Cd(II), V(V), Zr(IV), Pd(II), Sn(IV), Pb(II), Ag(I), which can react with QADMAP to form color chelates. To examine the selectivity of this method, the interference of foreign ions was investigated. When 5.0 ml of 1.0×10^{-4} mol/l QADMAP was used, for 20 μg/l of Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and Mn(II), respectively, the tolerance amount with an error of $\pm 5\%$ was 2000 μg/ml for Cd(II), V(V), Pd(II), Pb(II) and 800 μg/ml for Ag(I), Zr(IV), Sn(IV). This method is highly selective.

4. Conclusion

This method is highly selective and highly sensitive. The metal-chelate can be preconcentrated by C₁₈ cartridge. The detection limits of this method reaches the ng/l level. Most foreign ions do not interfere with the determination. This is one of the

Table 3
Regression equation, coefficient and detect limit

Components	Regression equation	Linearity range (μg/l)	Coefficient	Detect limits (ng/l)	RSD (% , $n=10$)
Fe–QADMAP	$A=6.82 \times 10^4 C (\mu\text{g/l}) + 78.6$	0.2–800	$r=0.9996$	10	2.5
Co–QADMAP	$A=8.43 \times 10^4 C (\mu\text{g/l}) + 64.3$	0.2–800	$r=0.9995$	12	3.2
Ni–QADMAP	$A=6.52 \times 10^4 C (\mu\text{g/l}) - 81.5$	0.3–1000	$r=0.9995$	8	2.8
Cu–QADMAP	$A=7.18 \times 10^4 C (\mu\text{g/l}) - 62.5$	0.3–1200	$r=0.9992$	13	2.4
Zn–QADMAP	$A=5.63 \times 10^4 C (\mu\text{g/l}) + 105$	0.5–1500	$r=0.9994$	17	2.2
Mn–QADMAP	$A=4.52 \times 10^4 C (\mu\text{g/l}) - 57.3$	0.4–1500	$r=0.9996$	22	2.9

most sensitive and selective methods for the simultaneous determination of Fe, Co, Ni, Cu, Zn and Mn. By solid-phase extraction, the consuming of organic solvents in this method is much lower than that consumed in liquid–liquid extraction method, and has a low pollution for the environment. By using the microwave digestion, only small amount of the sample (0.25 g) was needed, and little time was consumed (to digest a set of samples only needed 6.0 min). The Waters SPE device can prepare 20 samples simultaneously. This method is rapid for the simultaneous preparing a large amount of samples. With photodiode array detector, each metal-chelate can be monitored at its maximum absorption wavelength and the peak purity can be identified. The results are more reliable than single wavelength detector. In a word, for the simultaneous determination of Fe, Co, Ni, Cu, Zn and Mn in tobacco, this method is highly sensitive, highly selective and highly rapid.

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