Rapid Screening of Ascorbic Acid, Glycoalkaloids, and Phenolics in Potato Using High-Performance Liquid Chromatography

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Evaluation of phenolic metabolism in potato tubers (*Solanum tuberosum*) would be facilitated by faster analytical methods. A high-throughput HPLC method was developed for the qualitative and quantitative determination in potato of numerous phenolic compounds, the sum of the glycoalkaloids chaconine and solanine, plus ascorbic acid. Following a fast extraction, HPLC run times of 12 min were achieved with the use of a monolithic RP C_{18} column. UV and MS analyses were used to characterize the phenolic complement in extracts from two white-fleshed varieties. Over 30 compounds were identified, some of which are thought to possess either nutritional value or are involved in plant disease resistance. This method is expected to be useful for germplasm mining and for varietal development programs in which large numbers of lines are generated.

KEYWORDS: Ascorbic acid; functional foods; flavonoids; kukoamines; potato; phenolics; plant disease resistance; glycoalkaloids; *Solanum tuberosum*

INTRODUCTION

Recent years have seen increasing interest in the emerging field of "functional foods", a concept in which foods are considered to contain compounds that can provide health benefits beyond meeting the needs of basic nutrition (*1*). The potato (*Solanum tuberosum*) is among the most consumed vegetables in the world and, thus as a staple consumed in quantity, possesses considerable potential to be developed as a source of dietary phytochemicals.

Historically, relatively little effort in plant breeding has been directed toward maximizing the total nutritional potential of crops, probably due in part to the technological difficulties of such an undertaking combined with a lack of knowledge about which phytochemicals confer health benefits. However, these limitations are fading with the increasing availability, affordability, and ease of use of sophisticated instrumentation along with the emergence of high-throughput analytical techniques and the success of medical studies in identifying numerous health-promoting phytochemicals (*2−4*).

Analysis of the molecular regulation of phenolic biosynthesis in tubers would be made easier by the availability of a high-throughput HPLC method of analysis. Potato phenolics are of interest because some phenolics are increasingly being shown to have health benefits, including resveratrol (*5*), chlorogenic acid (*6*), and many others (*7*). In addition to their potential health benefits, phenolics are also important for their role in the resistance of plants to diseases, pests (*8−10*), and other stresses (*11*).

We sought to develop a rapid phenolic method that would simultaneously measure glycoalkaloids and ascorbic acid in tubers. Potato is a major source of ascorbic acid in the Western diet (*12*), and it potentially could be further increased through germplasm enhancement. Like most members of the Solanaceae family, potatoes have glycoalkaloids that can be toxic at higher concentrations (*13*). Glycoalkaloid levels can vary considerably among cultivars, and varieties with total glycoalkaloid levels above 20 mg/100 g of potato are generally considered unacceptable (*14*). Typically, ascorbic acid, phenolics, and glycoalkaloids in potato are each extracted by separate procedures that can be laborious. After extraction, different quantification methods are generally used for each, whether with HPLC, spectrometry, or ELISA. The ability to screen breeding lines for glycoalkaloid levels simultaneously along with ascorbic acid and phenolic analysis would help eliminate unacceptable varieties early in the selection process and eliminate the need for a separate glycoalkaloid analysis. Maximizing the amount of information gained from a single fast HPLC run could facilitate development of new varieties with superior nutritional qualities.

We are interested in developing potatoes with enhanced nutritive value or improved plant disease resistance through the use of molecular and conventional approaches. As a first step toward these goals, a HPLC method was developed that in a short 12 min run resolved numerous potato phenolics and other

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Table 1. Columns Tested during Method Development

column name	size (mm)	particle size (μm)	source
Spherisorb ODS2	4.6 × 250	5	Waters
Zorbax SB-AQ	3 × 150	3.5	Agilent
Zorbax Bonus-RP	3 × 150	3.5	Agilent
Zorbax XDB RR	3 × 150	3.5	Agilent
Zorbax XDB RRHT	4.6 × 50	1.8	Agilent
Zorbax NH2	4.6 × 250	5	Agilent
Atlantis	3 × 150	5	Waters
YMC C30	4.6 × 250	5	Waters
Onyx	4.6 × 100	monolithic	Phenomenex

compounds. Over 30 compounds present in methanolic extracts of potato were identified or tentatively identified using LC-MS.

MATERIALS AND METHODS

Chemicals. Ethyl acetate, EDTA, and HPLC grade methanol were purchased from Fisher (NJ). Acetonitrile, acetic acid, formic acid, and trifluoroacetic acid (TFA) were purchased from J. T. Baker (NJ). Ammonium formate was from Fluka. HPLC grade water with a conductivity of 18 mΩ was prepared with a Barnstead E-pure purification system (Dubuque, IA). A representative range of phenolics found in plants was used for method development. Ascorbic acid, chlorogenic acid, caffeic acid, ferulic acid, *p*-coumaric acid, phloroglucinol, *p*-hydroxybenzoic acid, dl-tyrosine, dl-tryptophan, and solanine were purchased from Sigma (St. Louis, MO). Chaconine was purchased from Fluka (St. Louis, MO.). Protocatechuic acid (3,4-dihydroxybenzoic acid) and vanillin were obtained from Avocado Research Chemicals (Heysham, UK). Kaempferol, myricetin, vanillic acid, and quercetin dihydrate were purchased from MP Biomedicals (Solon, OH). Gallic acid and (+)-rutin trihydrate were obtained from Alfa Aesar (Ward Hill, MA).

All standards were prepared as stock solutions at 10 mg/mL in methanol except tyrosine and tryptophan, which were prepared in 0.1 N HCl, and solanine, which was prepared in 2.5% metaphosphoric acid. Stock solutions of the standard solutions were stored in darkness at −80 °C. Standard solutions remained stable at least 6 months in these conditions.

Sample Preparation. Field grown tubers from the cultivars Ranger Russet and Norkotah Russet were harvested and washed. Longitudinal slices ∼1 mm thick were taken from the top, bottom, and center of two tubers, immediately frozen in liquid N_2, and then homogenized. One tuber per replicate was used. These powdered samples were then freeze-dried and stored at −80 °C until use. Freeze-dried powder (200 mg) was placed into a 2 mL screwcap tube along with 0.9 mL of extraction buffer (50% MeOH, 2.5% metaphosphoric acid, 1 mM EDTA) and 500 mg of 1.0 mm glass beads. Tubes were shaken in a BeadBeater (Biospec, Bartelsville, OK) for 15 min at maximum speed and centrifuged for 5 min at 4 °C, and the supernatant was transferred to a clean tube. The remaining pellet was reextracted with 0.6 mL of extraction buffer and centrifuged. The supernatants were combined, centrifuged, and concentrated in a Speed Vac (Thermo Savant, Waltham, MA) prior to HPLC analysis. Samples were kept chilled at all times and not exposed to bright light.

HPLC Analysis. An Agilent 1100 HPLC system equipped with an on-line degasser, quaternary pump, refrigerated autosampler, column heater, and DAD, FLD, and MS detectors was used for sample analysis. Flow rates ranged from 0.4 to 2 mL/min and injection volumes from 2 to 10 μL, depending on the column. Column temperature was 35 °C. The columns listed in **Table 1** were examined for use in separating the extracts using multiple gradients and mobile phases. Optimized conditions for the Onyx column (**Table 1**) used a flow rate of 2 mL/min and gradient elution of 0−1 min 100% buffer A (10 mM formic acid, pH 3.5, with NH₄OH), 1−5 min 0−30% buffer B (100% methanol with 5 mM ammonium formate), 5−6.5 min 40−70% buffer B, 6.5−8.5 min 70−100% buffer B. UV detection was at 210, 244, 280, 320,

and 360 nm. This buffer allowed good selectivity and substantially shorter run times than mobile phases with a lower pH such as those containing TFA. The XDB−RR column was operated at 1 mL/min with the same buffers as the Onyx column, using a gradient of 0−1.5 min 0−4% buffer B, 1.5−5 min 10−25% buffer B, 5−10 min 35−65% buffer B, 10−15 70−90% buffer B, 15−16 min at 95% buffer B. The external standard method of calibration was used, with each curve prepared from 6 to 8 different concentrations of standard solutions.

Limit of detection and quantitation were based on a signal-to-noise ratio of 3 and 9, respectively. Neochlorogenic and cryptochlorogenic acids were quantitated as chlorogenic acid equivalents. Kaempferol-3-*O*-rutinoside and quercetin-3-*O*-glu-rut as rutin equivalents at A_{360}. Dihydrocaffeoyl polyamines as dihydrocaffeic acid equivalents at A_{210} but with no adjustment made for the polyamine part of the molecule (*15*).

MS Parameters. LC−MS analysis was conducted with an Agilent 1100 LC/MSD VL ion trap. Experiments were carried out with an ESI source in either positive or negative ion mode, depending on the compound being examined. The source was operated using 350 °C drying gas (N_2) at 12 L/min, 55 psi nebulizer gas (N_2), and the source voltage with a scan range of 50−1000 *m/z*. Automated MS^2 analysis was conducted using Agilent's SmartFrag software and ramped CID voltage of 1500−4500. Data analysis was performed using Agilent Chemstation software and ACD/MS Manager software (Advanced Chemistry Development Inc. Toronto, Canada).

RESULTS AND DISCUSSION

Tuber Extraction. Development of potatoes and other crops with enhanced levels of phytonutrients would be facilitated by the availability of high-throughput analytical techniques for compounds of interest. Many different HPLC methods are used for analysis of plant phenolic compounds (*16−19*). While these methods can yield good results, they are generally not high-throughput and may involve laborious extractions of large tissue amounts and have long HPLC run times that can be over 90 min in length (*20−23*).

A variety of diverse solvents are used to extract plant phenolics. We tested several solvents, including 80% acetone, 90% ethanol, and 50% methanol with 0.5% acetic acid or 80% methanol. Samples extracted with acidified 50% methanol had better recoveries of chlorogenic acid, the most abundant phenolic in potato and also the aromatic amino acid, tyrosine. Tyrosine is associated with undesirable browning in potatoes (*24*), thus the ability to quantitate it by this method is useful. Optimal extraction of freeze-dried tissue was obtained using two 15 min extractions with a mini-beadbeater96, which allowed 45 samples to be processed simultaneously. This method gave equivalent or superior results to other methods tested, including extractions with shaking of samples in the dark for 1−24 h at either room temperature or 4 °C or extractions in which tissue was boiled. This procedure would have additional utility if it could extract ascorbic acid along with potato phenolics. Ascorbic acid is often extracted with aqueous metaphosphoric acid solutions (*25*) in which the metaphosphoric acid inhibits enzymatic degradation of the ascorbic acid during extraction. The efficacy of our method was confirmed by comparing it to an extraction made using a typical ascorbic acid extraction buffer containing 5% metaphosphoric acid (*25*). Based on peak area, the relative amount of ascorbic acid recovered with the dedicated method (*25*) was 398 ± 31, whereas our procedure with the 50% methanol buffer with the addition of either 5% or 2.5% metaphosphoric acid gave 454 ± 3 and 468 ± 13, respectively. Poor recoveries were obtained with the 50% methanol extraction buffer and 1% metaphosphoric acid.

Sample spiking with pure standards was used to determine the recovery efficiency of this method for a representative

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Table 2. Calibration and LOD Information Using the Onyx Column with UV Detection[a]

compound	K'	K' repeatability SD ($n = 3$)	calibration linear range (ng)	R^2	LOD (ng/injection)	LOQ (ng/injection)	wavelength (nm)	CV% ($n = 9$)
ascorbic acid	0.30	0.0034	10–2000	0.9999	0.5	2	244	1.8
tyrosine	0.87	0.0045	50–5000	0.9999	2	6	280	2.2
tryptophan	4.17	0.0131	20–5000	0.9998	0.5	2	280	2.7
phenylalanine	2.75	0.0112	20–5000	0.9981	0.5	3	210	1.5
chlorogenic acid	5.30	0.0201	10–7000	0.9999	0.1	1	320	2.1
caffeic acid	6.01	0.0368	10–7000	0.9992	0.2	1	320	1.9
ferulic acid	7.58	0.0425	10–2000	0.9997	0.2	1	320	3.2
rutin	7.97	0.0441	50–7000	0.9997	2	5	360	3.3
solanine + chaconine	8.63	0.0635	100–10000	0.9975	20	60	210	3.0

[a] LOD, limit of detection (ng/injection). LOQ, limit of quantitation. K', capacity factor. CV%, coefficient of variation percentage.
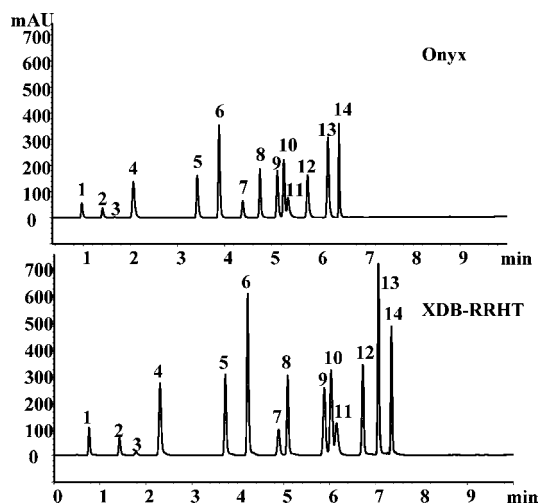

Figure 1. HPLC analysis of standards detected at 280 nm and separated on an Onyx or XDB-RRHT column. Flow rate was 2 and 1 mL/min for the Onyx and XDB columns, respectively. Peaks: 1, ascorbic acid; 2, tyrosine; 3, phloroglucinol; 4, gallic acid; 5, protocatechuic acid; 6, tryptophan; 7, p-hydroxy benzoic acid; 8, chlorogenic acid; 9, vanillic acid; 10, caffeic acid; 11, salicylic acid; 12, vanillin; 13, p-coumaric acid; 14, ferulic acid.

phenolic acid, flavonoid, glycoalkaloid, ascorbic acid, and amino acid. Good recoveries were observed for ascorbic acid (84.4% ± 2.7), tyrosine (99.4% ± 2.7), chlorogenic acid (90.0 ± 5), chaconine (97.5 ± 5.6), and rutin (98% ± 2.5).

Column Selection. Nine columns were tested (**Table 1**) during the process of selecting one that would be suitable for high-throughput analysis, giving rapid separation with low column bleed in a buffer suitable for LC−MS analysis. Good separation of phenolics was obtained with the XDB RR column, but ascorbic acid eluted in the void volume when using conditions suitable for separation of phenolic compounds. Retention of polar compounds on reverse-phase columns can sometimes be increased by reducing the amount of organic modifier in the mobile phase. However, reducing the methanol concentration below 2% resulted in column phase collapse or poor reproducibility. C_{30} columns are reported to be more resistant to phase collapse (*26*), and the tested column gave good separation of ascorbic acid and phenolics but required a rather lengthy run time of 30 min. Three columns (the Zorbax Bonus-RP, Zorbax SB-AQ and Atlantis) were subsequently tested because they are reported to have superior retention of polar compounds and are suitable for 100% aqueous mobile phases. The SB-AQ and Atlantis columns gave good resolution of ascorbic acid and phenolics, but the shortest run times achieved were about 30 min.

Better results were obtained with the final two columns tested (Onyx and XDB-RRHT). The double-endcapped XDB-RRHT column with an 80 Å pore size, 10% carbon load, and 1.8 μm particle size had the best sensitivity and peak sharpness of all the tested columns and a short run time of 20 min including reequilibration (**Figure 1**). However, this column was less resistant to fouling by the relatively crude potato extracts than was the Onyx column.

The endcapped monolithic Onyx column with an 18% carbon load gave a good separation of the 14 standards injected in less than 7 min (**Figure 1**). This column had low MS bleed, and the low back pressure allowed higher flow rates. Including reequilibration, total run time of potato extracts for the Onyx column was 12 min. Monolithic columns are made from a single piece of porous silica rod and have advantages over particle-based silica columns such as higher flow rates at lower back pressures. Monolithic columns are reportedly fairly robust and accommodating to relatively "dirty" biological samples, adding to their suitability for high-throughput analysis of plant extracts. After 500 injections of potato extracts, no obvious decline in column performance was observed. The capacity factors of 10 compounds separated on the Onyx column were determined (**Table 2**) and had good repeatability. For most compounds the limit of detection (LOD) using UV was less than a nanogram per injection (**Table 2**). The glycoalkaloids solanine and chaconine had an LOQ of 60 ng with UV detection at 210 nm. The linear calibration range exceeded 3 orders of magnitude for all compounds examined.

Analysis of Tuber Extracts. Compounds present in tuber extracts of the white-fleshed potato cultivars Ranger Russet and Russet Norkotah were separated on Onyx or XDB-RRHT columns and analyzed with an ion trap mass spectrometer and with DAD using five different wavelengths. Both the Onyx and XDB-RRHT columns were used initially because each gave good results, and we wanted to determine if after repeated use one might prove to be more suitable than the other. **Figure 2A** shows the chromatograph collected at 280 nm from a Ranger Russet extract, while the MS TIC current is shown in **Figure 2B**. Over 30 compounds were identified or tentatively identified (**Table 3**). Numerous very low abundance unidentified peaks were also detected with DAD, whereas the greater selectivity and sensitivity of MS detection revealed the presence of well over 100 peaks. Peak assignments were made based on UV and mass spectra and retention time comparisons to those of pure standards when available. The three aromatic amino acids (phenylalanine, tyrosine, and tryptophan) were all readily detected in extracts, with tyrosine being the most abundant (**Table 4**). The elution order of tryptophan and neochlorogenic acid was reversed between the two columns (**Figure 2A**). The
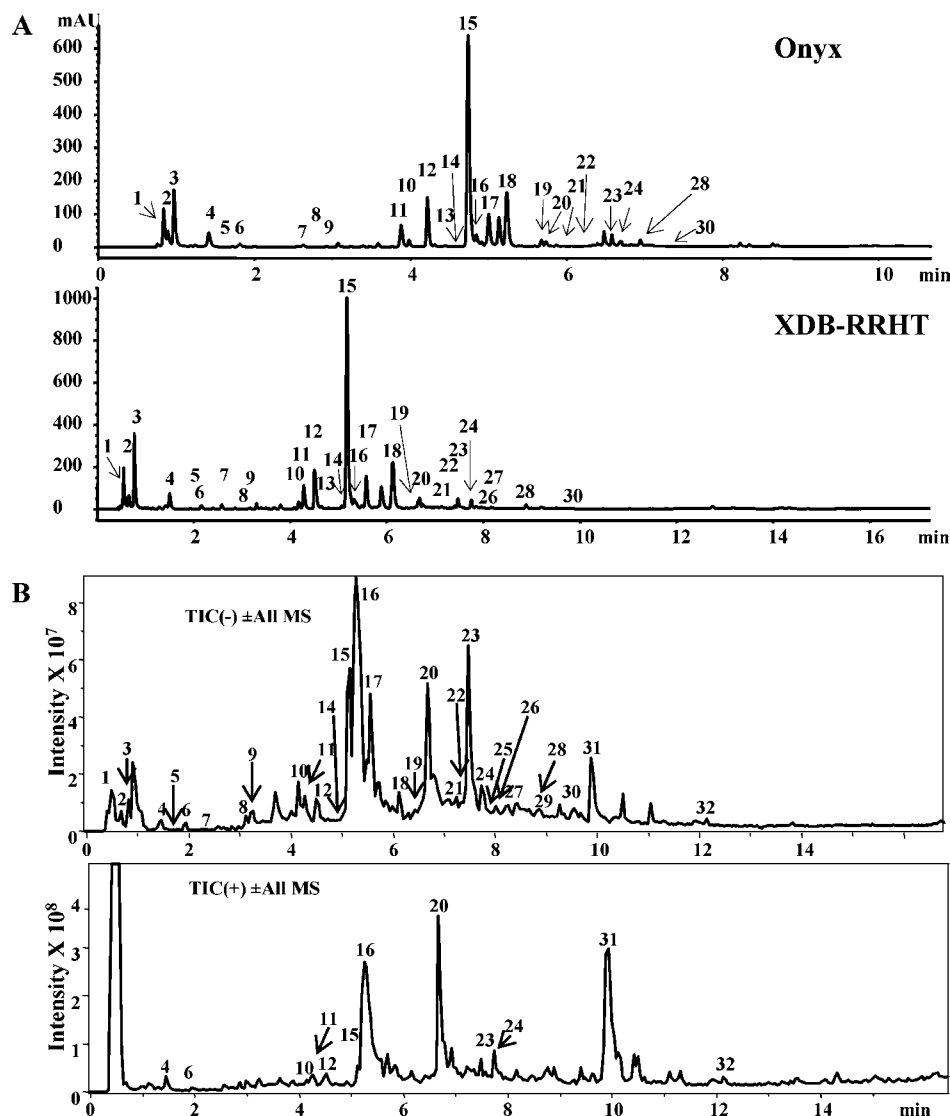
Figure 2. (**A**) Ranger Russet tuber extracts separated on an Onyx or XDB-RRHT column. HPLC chromatographs collected at 280 nm. (**B**) Negative and positive ion ESI LC-MS mass spectra of Ranger Russet extracts separated on the XDB-RRHT column. TIC is shown. Peak assignment is listed in **Table 3**.

salicylic acid glucoside (**14**) in extracts was identified with MS analysis, whereas the aglycone was detected with fluorometric detection but was below the limit of detection by MS. Salicylic acid is a key regulator of plant defense responses. Although measurement of salicylic acid in tubers requires separate fluorometric detection, the ability to measure salicylic acid glucoside is useful because this is the form plants store salicylic acid and this pool is much bigger than the aglycone pool. We have shown that in potato, SAG can comprise over 99% of the total salicylic acid (*27*). Thus, this screening method would readily detect germplasm with unusually high basal levels of total salicylic acid, because the contribution of free salicylic acid to the total salicylic acid pool is minimal. The mass spectra of **8** was consistent with it being either protocatechuic acid glucoside or gentisic acid glucoside, but the UV spectra identified it as gentisic acid glucoside (*28*), the glucosylated form of a phenolic compound also involved in plant disease resistance (*29*).

We find that after hundreds of injections, qualitative and quantitative analyses of extracts are reproducible allowing the amount of various compounds to be meaningfully compared among extracts from different varieties. This is also true for compounds which cannot be quantified due to unavailability

of pure standards. Coefficients of variation based on peak area were determined for three such compounds listed in **Table 3**, a caffeoyl spermine derivative (peak 6), a salicylic acid glucoside, and a ferulic acid amide (**24**); the CV% was 2.8, 1.2, and 1.2, respectively.

Caffeic Acid Derivatives. Chlorogenic acid (5-CQA, 5-*O*-caffeoyl quinic acid, **15**) was by far the most abundant phenolic, as has previously been reported for potato (*30*). Chlorogenic acids are esters of *trans*-cinnamic acids and quinic acid, and multiple forms exist. Chlorogenic acid (5-CQA) was present at 1.0 and 2.2 mg/g dry wt for Ranger and Norkotah, respectively (**Table 4**), and in Norkotah constituted over 60% of the total soluble phenolics. Neochlorogenic (3-*O*-caffeoyl quinnic acid; **10**) and cryptochlorogenic acid (4-*O*-caffeoyl quinnic acid; **17**) were identified on the basis of UV spectra and fragmentation patterns previously established for dicaffeoyl quinic acid isomers (*31*). Combined, they were present at concentrations between 15 and 20% of 5-CQA. Lesser amounts of another chlorogenic acid isomer (**5**) was identified as 1-*O*-caffeoyl quinic acid, and this had a similar mass spectra as 5-CQA but was less hydrophobic than the authentic 5-CQA standard (*31*). All four chlorogenic acid isomers yielded the deprotonated quinic acid ion at *m/z* 191 [M − H − caffeic acid]⁻

Table 3. List of Compounds Identified in Potato by LC–MS[a]

peak	tR (min)	name	m/z [M − H]⁻	MS² (relative abundance) % (negative mode)
1	0.6	quinic acid derivative	379	289(100), 217(32), 191(19)
2	0.7	caffeoyl-D-glucose	339	179(100), 161(26), 177(12), 143(19)
3	0.78	ascorbic acid*	175	115(100), 87(15), 71(6)
4	1.42	tyrosine*	180	163(100), 119 (21), 93(16)
5	1.8	1-*O*-caffeoyl quinic acid	353	191(100), 179(32), 135(9)
6	1.9	caffeoyl spermine derivative	611	306 (100), 272 (53), 254 (23), 179 (14), 288 (12)
7	2.6	caffeic acid derivative	371	353 (100), 191(31), 173(18), 179(7)
8	3.1	gentisic acid-glucoside	315	153 (100), 108 (22), 109 (21),
9	3.2	phenylalanine*	164	147(100), 148(6), 72(2)
10	4.1	neochlorogenic acid	353	191(100), 179(40), 135(19)
11	4.2	tryptophan*	203	159(100), 116(58), 142(18), 186(6)
12	4.5	caffeoyl putrescine	249	135 (100), 179(0.2), 161 (0.1)
13	4.7	3-*O*-caffeoyl, 5-*O*-feruloylquinic acid	529	191(100), 367(78), 407(13)
14	4.8	salicylic acid-glucoside	299	137(100), 138(10)
15	5.2	chlorogenic acid*	353	191(100),179(14), 161(2),135(2)
16	5.4	bis(dihydrocaffeoyl) spermine	529	365 (100), 407(16)
17	5.5	cryptochlorogenic acid	353	173(100), 179(53), 191 (13), 135 (2)
18	6.0	caffeic acid*	179	107(100), 75(60)
19	6.6	5-*O*-feruloyl quinic acid	367	191(100), 193 (4)
20	6.7	bis(dihydrocaffeoyl) spermidine	472	308(100), 350(22)
21	7.1	quercetin-3-*O*-glu-rut	771	300(100), 301(47), 609 (15), 591(54)
22	7.3	caffeoyl methyl quinate	367	179(100),135(99), 191(13)
23	7.4	tris(dihydrocaffeoyl) spermine	693	529(100), 365 (25), 407 (17)
24	7.7	ferulic acid amide	310	135(100), 161 (69), 252 (25), 295(35)
25	7.9	4,5-di-*O*-caffeoyl quinic acid	515	353(100), 191(19)
26	8.1	N^1,N^4,N^8-tris(dihydrocaffeoyl) spermidine	636	472(100), 514(18), 350(16), 308(18)
27	8.2	rutin*	609	301 (100), 271(13), 343 (13)
28	8.8	kaempferol-3-*O*-rutinoside	593	285(100), 327 (8)
29	8.9	N^1,N^4,N^8,N^{12}-tetra(dihydrocaffeoyl) spermine	857	693(100), 529(40), 517(19), 407(10), 365 (7), 725 (6)
30	9.8	quercetin*	301	179(100), 151(86), 273(22)
31	9.9	solanine + chaconine*	866 + 850	704(100), 558(2)
32	12.1	quercetin dimethyl ether	329	171(100), 125(33), 210(32)

[a] An asterisk (*) designates compounds identified on the basis of MS and coelution of pure standard. The remaining compounds are identified on the basis of MS.

Table 4. Quantitation of Select Compounds Present in Tuber Extracts

peak	compound	Norkotah (mg/g dry wt)	SD (n = 3)	Ranger (mg/g dry wt)	SD (n = 3)
3	ascorbic acid	0.36	0.069	0.78	0.13
4	tyrosine	0.63	0.063	0.41	0.0051
6	caffeoyl spermine derivative	0.070	0.0085	0.035	0.0027
9	phenylalanine	0.46	0.068	0.30	0.053
10	neochlorogenic acid	0.099	0.02	0.029	0.0078
11	tryptophan	0.12	0.02	0.083	0.020
12	caffeoyl putrescine	0.022	0.0018	0.11	0.036
15	chlorogenic acid	2.2	0.23	1.0	0.14
16	bis(dihydrocaffeoyl)spermine	0.28	0.048	0.091	0.016
17	cryptochlorogenic acid	0.27	0.035	0.16	0.022
18	caffeic acid	0.062	0.014	0.19	0.023
20	bis(dihydrocaffeoyl) spermidine	0.024	0.00088	0.048	0.013
21	quercetin-3-*O*-glu-rut	trace[a]		0.025	0.0083
23	tris(dihydrocaffeoyl) spermine	0.054	0.0035	0.084	0.023
26	tris(dihydrocaffeoyl) spermidine	trace		0.0054	0.0015
27	rutin	0.0046	0.00066	0.026	0.0089
28	kaempferol-3-*O*-rutinoside	0.0079	0.00058	0.0067	0.00059
31	solanine + chaconine	0.33	0.032	0.37	0.066

[a] Trace = below LOQ.

in both MS and MS² (**Table 3**) and a *m/z* 179 [caffeic acid − H]⁻ ion fragment. Substantial amounts of the possible chlorogenic acid precursor, caffeoyl-D-glucose (**2**) was also present (*32*). Another identified chlorogenic acid was 5-*O*-feruloyl quinic acid (**19**; *m/z* 367 [M − H]⁻) (*33*). A chlorogenic acid (**25**, *m/z* 515 [M − H]⁻) was detected with a MS² base peak at *m/z* 353 [M − caffeic acid − H]⁻ and was differentiated from other dicaffeoyl quinic acid isomers by the absence of a MS² fragment at *m/z* 335 and the presence of a MS³ [515 → 353] base peak of 173, which identified it as 4,5-di-*O*-caffeoyl quinic

acid (*31*). The aglycone caffeic acid (**18**, *m/z* 179 [M − H]⁻) was also present, with Ranger Russet having about three times as much as Norkotah (**Table 4**). Several hydroxycinnamic acid amides were detected. One caffeoyl amide with a deprotonated molecular ion of *m/z* 249 was identified as *N*-caffeoyl putrescine (**12**). Compound **24** was tentatively identified as a ferulic acid derivative containing a nitrogen, possibly a feruloylamide. Compounds **6** and **7** were unidentified caffeic acid derivatives. Compound **13** was a caffeoyl feruloyl quinic acid isomer, and the MS² base peak of 353, prominent secondary peak of *m/z*
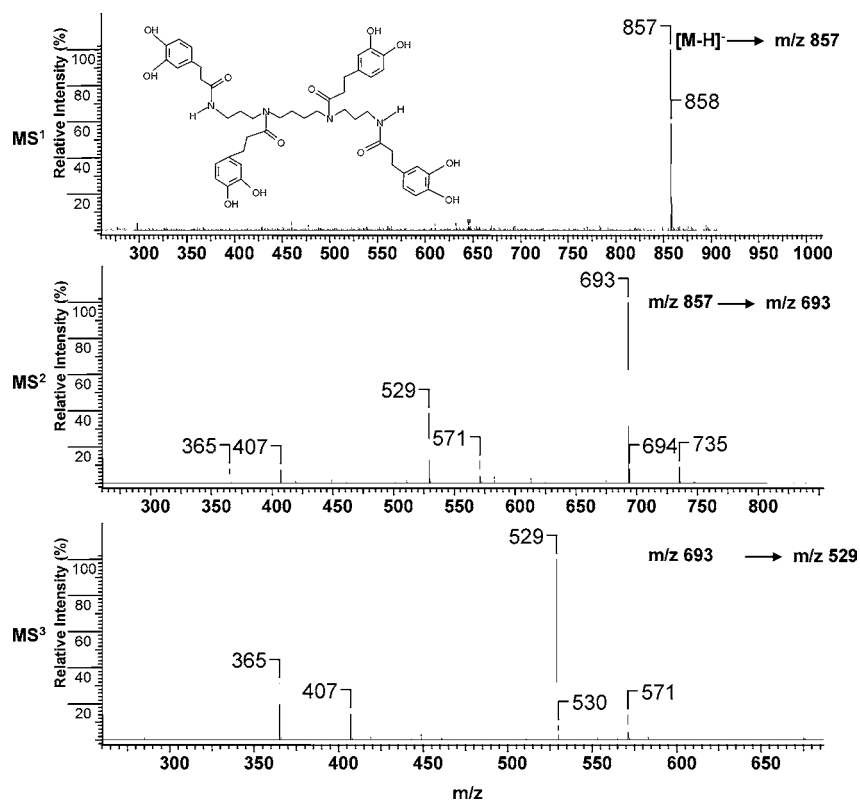
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Figure 3. Negative ion ESI LC–MS of **29**, N^1,N^4,N^9,N^{12}-tetra(dihydrocaffeoyl)spermine. MS, MS^2, and MS^3 data are shown.

367, and MS^3 base peak of 191 suggested that this compound was most likely 3-O-caffeoyl,5-O-feruloyl quinic acid (*33*).

Polyamine Derivatives. Additional compounds with a caffeoyl component, dihydrocaffeoyl polyamines, were identified based on their UV and mass spectra. Recently Parr et al. (*15*) identified four kukoamines (dihydrocaffeoyl polyamines) in potato extracts, which was the first time these compounds had been identified in any other plant besides a Chinese medicinal plant. Compound **16** was an *N,N'*-bis(dihydrocaffeoyl) spermine with a pseudomolecular ion of 529 [M − H]$^−$. An *N,N'*-bis-(dihydrocaffeoyl) spermidine with a pseudomolecular ion of 472 [M − H]$^−$ was also present (**20**). Compound **23**, with a pseudomolecular ion of 693 [M − H]$^−$, was a tris(dihydrocaffeoyl) spermine, and the MS^2 mass spectra showed a base ion of 529, corresponding to the loss of a dihydrocaffeoyl group. These three dihydrocaffeoyl polyamines in the two potato varieties we examined are possibly the same as previously identified kukoamines (*15*), but in the absence of pure standards, we could not definitively identify which of the several possible isomers they are. Only one relevant isomer is possible for **26**, which was identified as N^1,N^4,N^8-tris(dihydrocaffeoyl) spermidine with ions corresponding to loss of one or two of the dihydrocaffeoyl groups present at *m/z* 472 and 308 in the MS^2 spectra. Furthermore, **29** (**Figure 3**) was identified as $N^1,N^4,$ N^9,N^{12}-tetra(dihydrocaffeoyl) spermine (*m/z* 857, [M − H]$^−$), which is the only possible relevant isomer and was previously not definitively confirmed in potato. Loss of a dihydrocaffeoyl moiety yielded the base peak of *m/z* 693 in MS^2. Further fragmentation of *m/z* 693 led to loss of another dihydrocaffeoyl group, resulting in the base peak of *m/z* 529 in MS^3. The *m/z* 365 peak corresponded to loss of a third dihydrocaffeoyl group. The ions at *m/z* 735 and 571 arise from loss of $C_7H_6O_2$ from dihydrocaffeoyl groups in MS^2 and MS^3, respectively (**Figure 3**). Numerous additional spermines were detected, including some that appeared to be feruloyl conjugates.

The role of polyamines in tubers is unclear, but some are implicated in pathogen resistance (*34*). These kukoamines were found primarily in the tuber periderm (data not shown), which would be consistent with a role in defense.

Flavonoids. Over 4500 flavonoids exist and some of these polyphenol compounds have potential health benefits. Several flavonol glycosides were detected in the two white-fleshed cultivars examined. The presence of compounds with UV spectra showing a shoulder at 296 nm and maxima at 352 nm suggested that several quercetin derivatives were present. Compound **27** was identified as the flavonol glycoside rutin (quercetin-3-O-rutinoside, *m/z* 609, [M − H]$^−$) comprised of quercetin and the disaccharide rutinose. The MS^2 ion at *m/z* 301 results from loss of the disaccharide yielding the quercetin aglycone (**Table 3**). UV and MS analyses also detected a quercetin-containing compound (**21**) with *m/z* 771 [M − H]$^−$. MS^2 fragmentation of this compound showed a peak at *m/z* 609, which corresponds to rutin, resulting from the loss of a terminal hexosyl residue (*m/z* 162, [M − H − glucose]$^−$) from the original compound. A *m/z* 301 peak was indicative of the deprotonated quercetin aglycon. We were unable to determine whether this compound was quercetin 3-O-rutinoside-7-O-glucoside or quercetin-3-O-glucosylrutinoside, but the absence of a MS^3 ion at *m/z* 462 suggests the latter compound is the more likely candidate (*35, 36*). Trace amounts of additional quercetin flavonols were detected, but quantities were too small to generate informative MS spectra. Quercetin-3-O-diglucoside was previously reported in potato (*36*) but was not detected in the two varieties we sampled. Kaempferol-3-O-rutinoside (**28**, *m/z* 593 [M − H]$^−$) was identified, with MS^2 fragmentation generating the kaempferol aglycone at *m/z* 285 [M − H − rutinose]$^−$.

Glycoalkaloids. The two major glycoalkaloids in potato are α-solanine and α-chaconine, which together comprise approximately 95% of tuber glycoalkaloids (*37*). Solanine and chaconine resolved as separate peaks on the XDB-RRHT

Rapid Screening Using HPLC

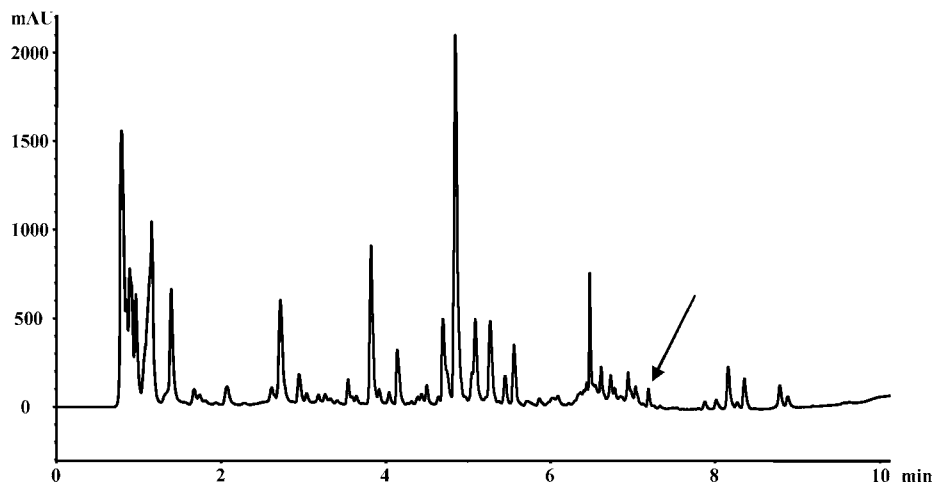*J. Agric. Food Chem.,* Vol. 54, No. 15, 2006 **5259**

Figure 4. Ranger Russet tuber extract separated on an Onyx column. HPLC chromatograph collected at 210 nm. The combined solanine and chaconine peak is indicated by the arrow.

column and a single peak on the Onyx column. The limit of detection for solanine or chaconine was about 20 ng per injection using UV detection and less than 5 ng with ESI−MS. Although MS detection is more sensitive and selective for these two glycoalkaloids, their abundance in white-fleshed potatoes could also be monitored using A_{210} UV detection (**Figure 4**). Solanine and chaconine amounts exceeding 20 mg/100 g FW would be readily detected. Both of these white-fleshed varieties had between 300 and 400 μg/g dry wt of solanine and chaconine (**Table 4**). Preliminary analysis of wild potato species with this method resolves numerous additional glycoalkaloids (data not shown).

Conclusion. A method suitable for screening a large number of biological samples has unique requirements relative to methods designed for analysis of fewer or less complex samples. The protocol described in this paper is easy to use, rapid, inexpensive, and generates a substantial amount of qualitative and quantitative information per sample, all criteria conducive to high-throughput analysis of plant samples. We have also used this procedure successfully with tomato samples, thus the utility of this method may extend to other plants besides potato. It should be noted that we used MS analysis primarily for peak identification and purity analysis. Although many more compounds are detected with MS than DAD, all of the compounds in **Table 3** were readily detected with a diode-array detector (**Figures 2A** and **4**). Thus, for screening, this method may also be useful for labs using only a traditional HPLC-DAD system, without MS instrumentation. Whereas coelution of peaks with different masses is generally more easily handled with LC−MS, coelution could be a potential problem if only using DAD. However, careful use of peak purity analysis and spectral analysis with DAD combined with the use of a baseline from extracts from Russet Burbank or Russet Norkotah as described in this paper should yield good results and enable ready detection of potential problems from coeluting peaks. Our ongoing work using combined MS and DAD with numerous white- and yellow-fleshed varieties suggests that DAD detection alone would work well as a screening tool when using the above caveats.

Similarly, HPLC-DAD is more widely available and less expensive than a capillary and robotics based approach, which presumably would allow even faster analysis. High-throughput analysis of potato phenolics should facilitate study of molecular regulation of phenolic biosynthesis, facilitate analysis of how plant phenolic profiles change in response to various stimuli,

and also allow approaches not previously feasible in varietal development programs.

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