Application Note

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µSPEed | Application Note 2015

µSPEed extraction and UHPLC-PDA analysis of polyphenols in teas

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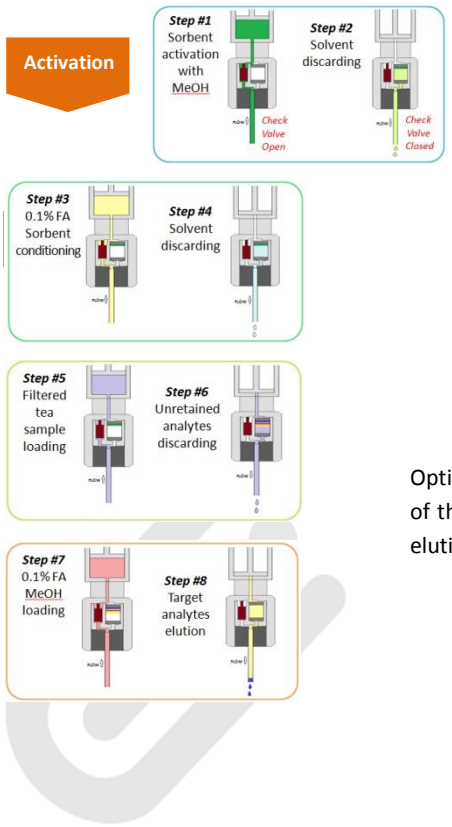
INTRODUCTION

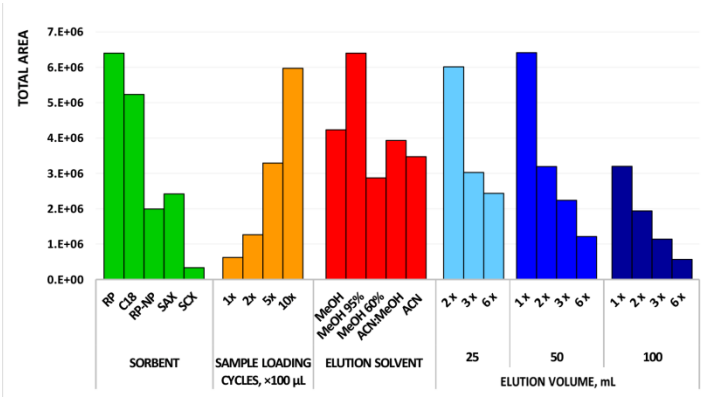
Polyphenols are widespread constituents of several foods matrices, including fruits, juices and beverages, as tea and wine, being partially responsible for their overall organoleptic properties. Despite their wide distribution, the health effects of dietary polyphenols have come to the attention of nutritionists only in recent years. Growing epidemiologic evidences link polyphenols to protective effects against several highly prevalent human diseases (as oncologic, cardiovascular and neurodegenerative diseases).

The objective of this study was to evaluate the performance of an innovative analytical approach, µSPEed, to isolate phenolics compounds from teas. µSPEed is a simple and fast extraction procedure using small particle sorbents (3µm or less, 20 x smaller than SPE particles), enabling a more efficient separation of the target analytes from interfering matrix.

PROCEDURE

µSPEed Extraction Process





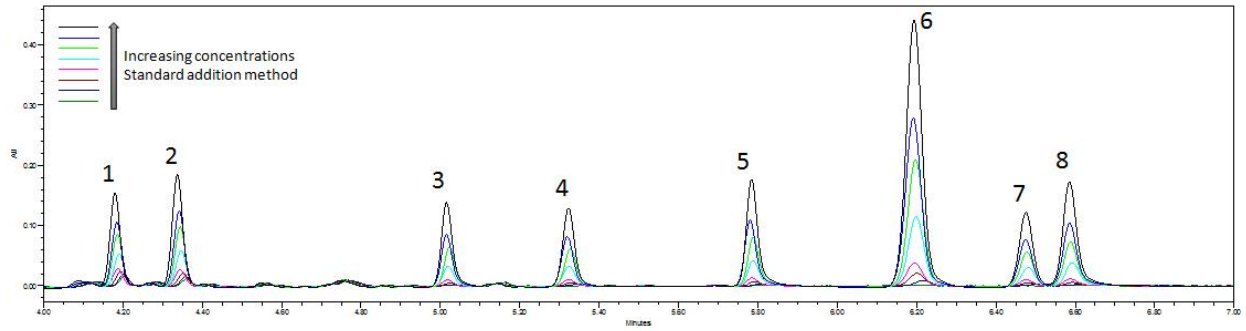
Optimization of the best µSPEed experimental conditions: selection of the best sorbent **(A)** , optimal sample loading condition **(B),** best elution solvent **(C)** and respective optimal elution volume **(D)**.

Chromatography

UHPLC system using a Waters Ultra Pressure Liquid Chromatographic Acquity system equipped with a quaternary solvent manager (QSM), an Acquity sample manager (SM), a column heater, a degassing system and a photodiode array (PDA) detector. Acquity BEH C18 analytical column (2.1 mm×50 mm, 1.7 µm particle size)at 35 °C. Binary mobile phase of solvent A (0.1 % FA) and solvent B (ACN) were combined in the following gradient: 90 % A (0 min), 85% A (1.50 min), 72% A (3.0 min), 66% A (4.0 min), 56% A (6.7 min), and 90 % A (7.0 min). The flow rate was set between 200 and 250 µL/min (250 µL/min - 0 min, 220 µL/min - 3 min, 200 µL/min - 6.7 min and 250 µL/min - 7 min), originating a maximum back pressure of 4700 psi. Injection volumes 2µL and the samples were kept at 20°C during the analysis. The PDA wavelength was fixed in 270, 307 and 370 nm.

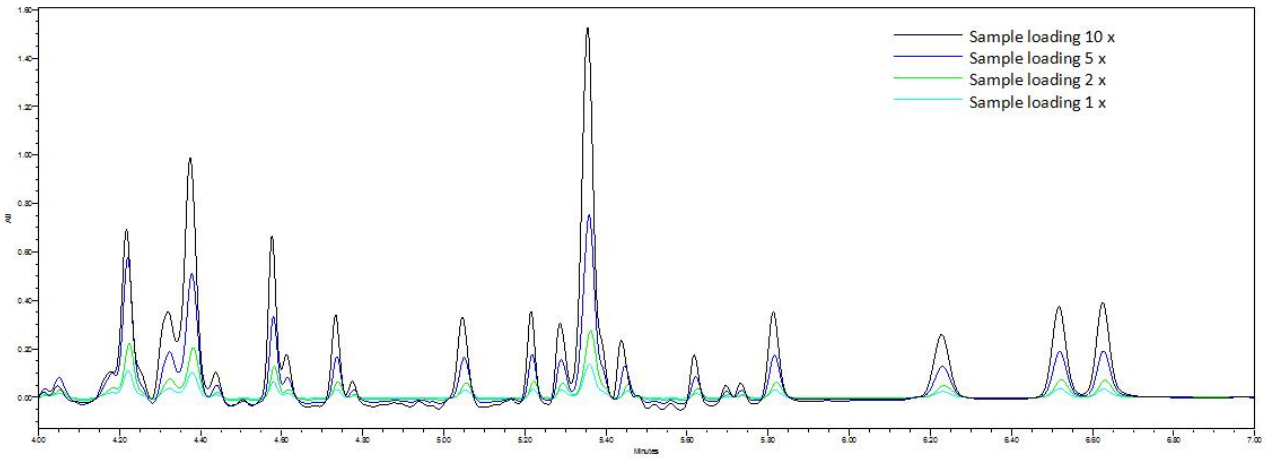
RESULTS

Recovery Linearity - µSPEed/UHPLC-PDA analysis

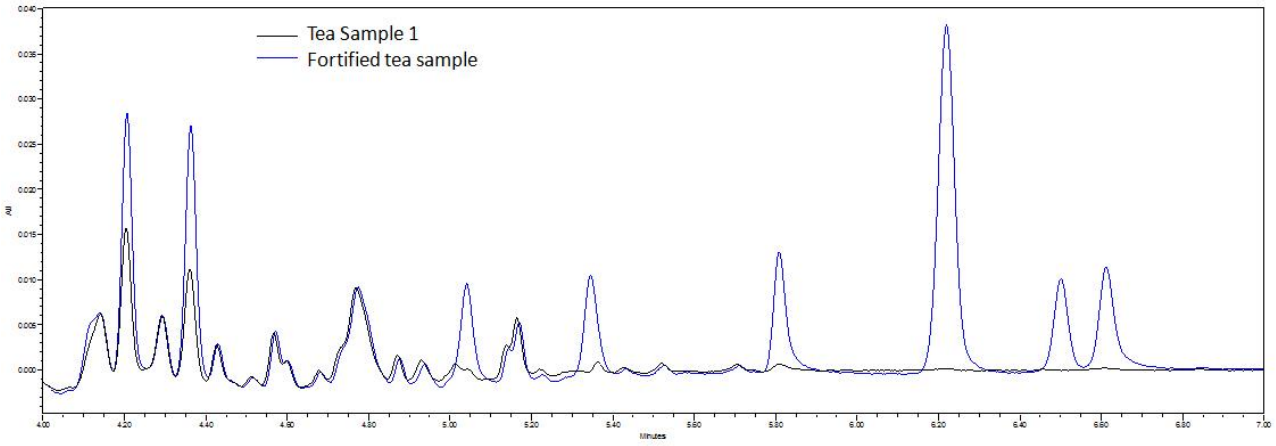


Chromatogram of a mixture of phenolics compounds standards upon µ-SPE extraction and UHPLC analysis: 1 – Rutin, 2 – Quercetin-3-glucoside, 3 – Myricetin, 4 – trans-Resveratrol, 5 – Quercetin, 6 – Cinnamic acid, 7 – Narigenin, 8 – Kaempferol

Sample Loading - µSPEed/UHPLC-PDA analysis



Tea Sample -µSPEed/UHPLC-PDA analysis



Validation of µSPEed/UHPLC-PDA analysis

#	Phenolic Compound	RT	λ_{max}	LDR (µg/mL)	R^2	LOD (µg/mL)	LOQ (µg/mL)	ME (%)	Recovery (%)	Precision (% RSD) Intraday (*n*=12)	Precision (% RSD) Interday (*n*=36)
1	Rutin	4.24	270	0.2 - 20	0.998	0.017	0.051	105.8	98.0	0.4	1.1
2	Quercetin-3-glucoside	4.39	270		0.998	0.005	0.014	97.6	97.0	0.5	0.9
3	Myricetin	5.08	370		0.997	0.004	0.013	95.0	95.6	0.5	1.7
4	trans-Resveratrol	5.39	307		0.998	0.004	0.013	96.9	98.6	0.4	1.1
5	Quercetin	5.84	270		0.998	0.004	0.012	98.1	100.7	0.2	1.7
6	Cinnamic acid	6.25	270		0.998	0.008	0.024	95.7	93.3	0.5	1.8
7	Narigenin	6.54	370		0.998	0.004	0.011	94.4	94.6	0.3	0.7
8	Kaempferol	6.66	370		0.997	0.005	0.014	98.2	97.6	0.5	1.2

Validation parameters for the µSPEed/UHPLC-PDA analysis of the selected phenolics compounds: RT – retention time; LDR – linear dynamic range; Max abs – Maximum absorbance values obtained with PDA detection; R^2 – correlation coefficient; LOD – limits of detection; LOQ – limits of quantification; ME – Matrix effect; RSD – relative standard deviation.

Tea analysis

Phenolic Compound	Concentration (µg/mL)		
	Tea 1	Tea 2	Tea 3
Rutin	1.10	13.58	16.34
Quercetin-3-glucoside	0.66	17.71	9.24
Myricetin	0.21	0.26	0.14
trans-Resveratrol	0.80	-	-
Quercetin	0.06	0.05	0.08
Cinnamic acid	-	0.03	-
Narigenin	-	-	-
Kaempferol	0.12	0.03	0.07

Occurrence of the selected phenolics compounds in the teas analyzed

CONCLUSION

µSPEed was optimized for the extraction of 8 selected phenolics compounds from teas, being 100µL of filtered tea sample, loaded twice through the RP sorbent and elution with 100 L of MeOH 95% the best conditions.

µSPEed/UPLC-PDA analysis retrieved excellent analytical performance: good LODs and LOQs, good selectivity, linearity (r2 >0.99) and recovery (>93%); good intraday and inter-day precisions (RSD <5%) and negligent matrix effect. The methodology developed is fast and semi-automatic, involving minimal sample pretreatment and solvent usage, while allowing the rapid and simultaneous determination of 8 phenolics compounds in teas with high sensitivity.

The abundance of the selected phenolics compounds in teas encompass a broad range of concentrations, being rutin and quercetin-3-glucoside the most abundant.

ACKNOWLEDGEMENTS

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REFERENCE

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µSPEed Ordering Information

Part Number	Code	Description
µSPEed Cartridges		
01-10110	µSPEed, C18RPS-3µm/120Å (Pkt 10)	3µm/ 120Å ODS spherical silica packing with high acidic resistance suitable for general organic compound applications.
01-10115	µSPEed, Silica-3µm/120Å (Pkt 10)	3µm/120Å spherical bare silica packing. High purity silica for normal and hilic applications
01-10150	µSPEed, PS/DVB -3µm/ 300Å (Pkt 10)	3µm/ 300Å spherical, crosslinked polystyrene divinyl benzene
01-10151	µSPEed, PS/DVB RP-3µm/ 300Å (Pkt 10)	3µm/ 300Å Phenyl (RP) spherical, crosslinked polystyrene divinyl benzene
01-10155N	µSPEed, PS/DVB SAX-3µm/ NP (Pkt 10)	3µm/Non Porous SAX spherical, crosslinked polystyrene divinyl benzene
01-10156N	µSPEed, PS/DVB SCX-3µm/ NP (Pkt 10)	3µm/Non Porous SCX spherical, crosslinked polystyrene divinyl benzene