Electronic Supplementary Material (ESI) for Analyst.

SUPPLEMENTARY INFORMATION

Simultaneous multiplexed quantification of nicotine and its metabolites using surface enhanced Raman scattering.

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Table S1. Examples of some of the tertiary mixtures and how they were generated from 3 stock solutions of nicotine, cotinine and *trans*-3'-hydroxycotine. For these experiments the total molarity in each tube was constant at 1.10^{-5} M (10 µmol/L).

Sample number	Concentration (µmol/L)			Volume (µl) prepared from working stocks (1.10^{-5} M)		
	Nicotine	Cotinine	Trans	Nicotine	Cotinine	Trans
1	0	0	10	0	0	200
2	1	5	4	20	100	80
3	2	4	4	40	80	80
4	3	6	1	60	120	20
5	4	1	5	80	20	100
6	5	5	0	100	100	0
7	6	3	1	120	60	20
8	8	1	1	160	20	20
9	9	0	1	180	0	20
10	10	0	0	200	0	0
n	…	…	…	…	…	…

Table S2. Chemometric model predictions for nicotine, cotinine and *trans*-3'-hydroxycotinine. In these predictions three output nodes were used in the ANNs.

		Nicotine		Cotinine		*Trans*-3'-hydroxycotinine	
		Q^2	RMSEP	Q^2	RMSEP	Q^2	RMSEP
pH = 3	ANN	0.8691	9.7993	0.4470	19.9844	0.5697	17.6286
	K-PLS	0.8514	10.3858	0.4401	20.1457	0.5247	18.4952
pH = 10	ANN	0.9344	6.8851	0.7800	12.6056	0.7991	12.0441
	K-PLS	0.9294	7.1119	0.7741	12.7430	0.7901	12.2692
pH = 11	ANN	0.9198	7.6096	0.7734	12.7929	0.8172	11.4898
	K-PLS	0.8999	8.5718	0.7687	12.8987	0.8054	11.9001
Combined	ANN	0.9395	6.6094	0.8739	9.6424	0.8928	8.7998
	K-PLS	0.9279	7.2309	0.8684	9.8848	0.8887	9.0175

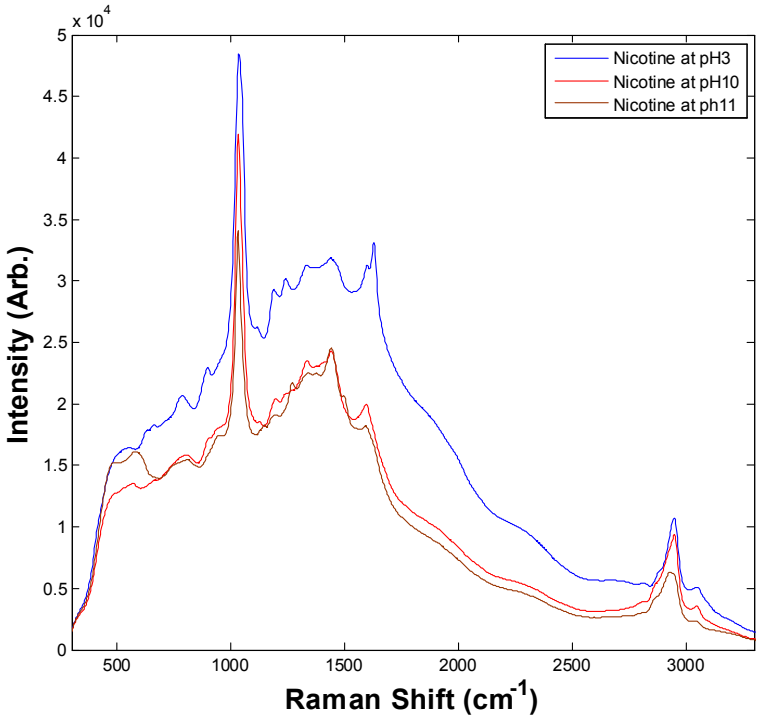

Figure S1. SERS spectra from nicotine collected at pH 3, 10 and 11.

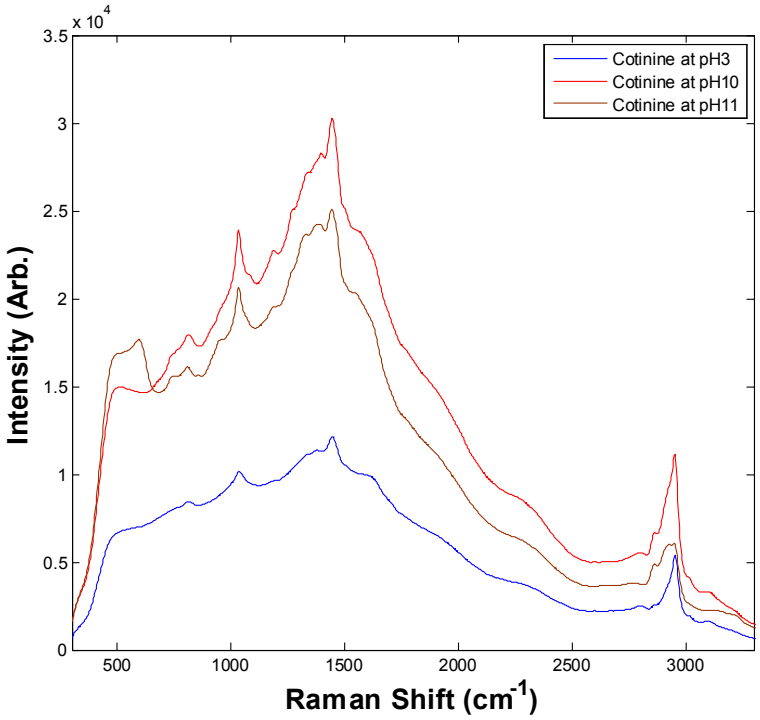

Figure S2. SERS spectra from cotinine collected at pH 3, 10 and 11.

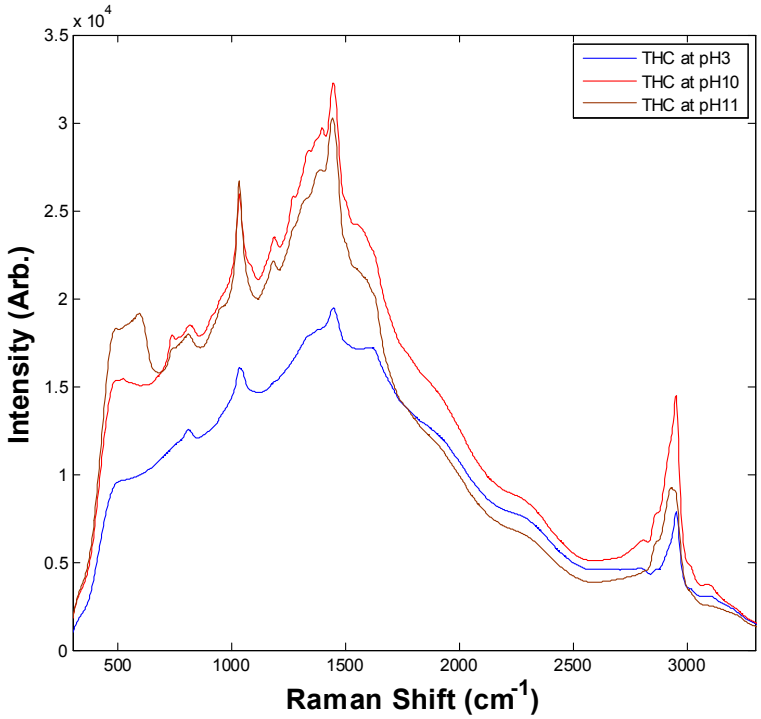

Figure S3. SERS spectra from *trans*-3'-hydroxycotinine collected at pH 3, 10 and 11.

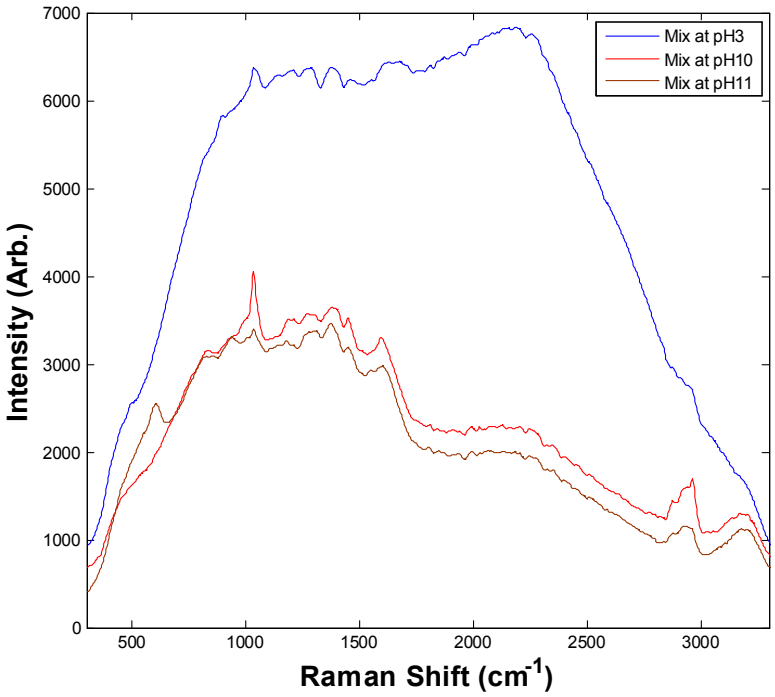

Figure S4. SERS spectra from a mixture of nicotine : cotinine : *trans*-3'-hydroxycotinine at a ratio of 60:20:20 collected at pH 3, 10 and 11.

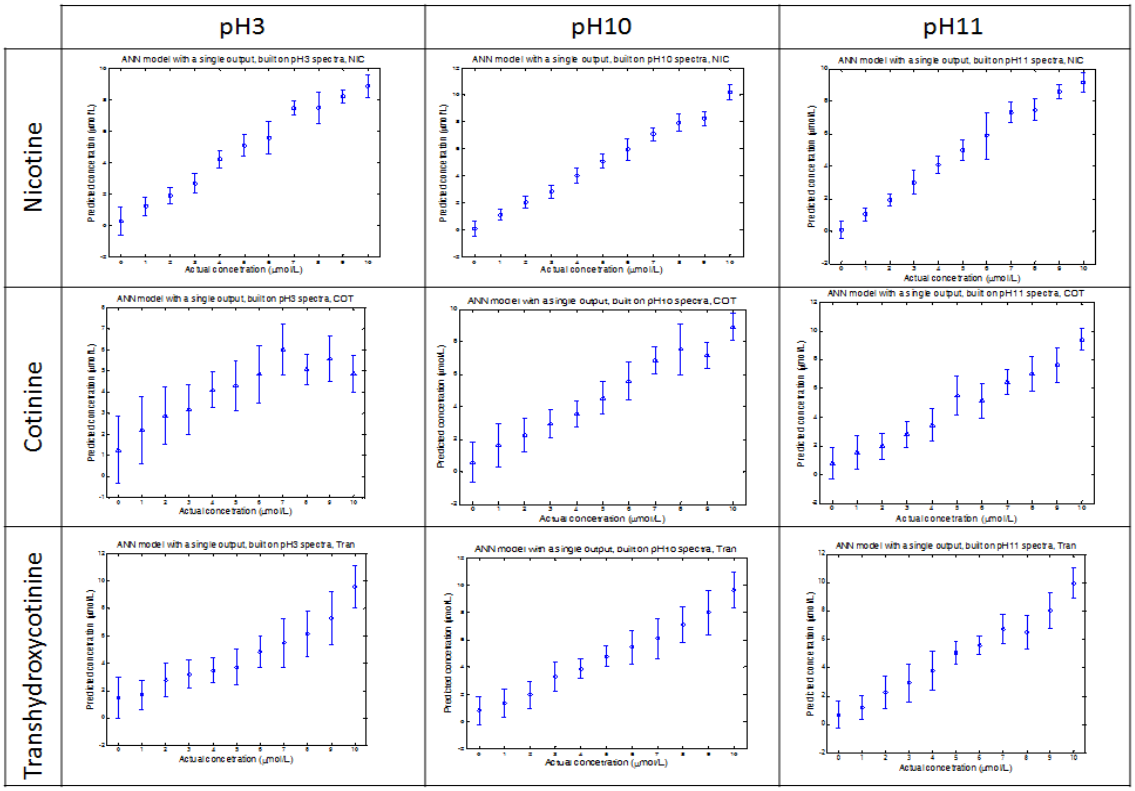

Figure S5. Chemometric model predictions from artificial neural networks (ANNs) for the nicotine, cotinine and *trans*-3'-hydroxycotinine. In this example 9 individual ANNs were constructed with SERS spectra generated from each of the 3 different pH conditions with and a single output was used for each of the three analytes. Points show the averages of the test data only with standard deviation error bars shown.