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A novel method for the simultaneous determination of five low calorie sweeteners in human urine

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The use of low calorie sweeteners (LCS) as substitutes for sugar in food and beverage products has increased as a strategy to reduce the energy density of the diet[(1)]. As food additives, LCS are assigned an acceptable daily intake (ADI) following examination of available safety and toxicological data. In Europe, member states are required to monitor consumption of LCS to ensure that the ADI is not being exceeded[(2)]. Traditional methods of monitoring are prone to error and as LCS pass through the body largely unchanged[(3–5)], the opportunity may exist to use a biomarker approach to investigate levels of exposure to LCS. Although analytical methodologies have been published describing the simultaneous determination of LCS in foodstuffs[(1)] as well as waste water[(6)], to date no such method has been described for use in human urine.

This project aimed to develop a novel method of simultaneously determining four LCS (acesulfame-K, saccharin, cyclamate, sucralose) and the excretory product of a relatively new sweetener, steviol glycosides (steviol glucuronide), in human urine. An LC-MS/MS method was developed to separate and quantify these LCS and steviol glucuronide. Sodium cyclamate d-11 (for cyclamate) and warfarin sodium (for acesulfame-k, saccharin, sucralose and steviol glucuronide) were used as internal standards. Method validation was carried out by assessing linearity and range, limits of detection (LOD), limits of quantification (LOQ), precision and accuracy. Preliminary validation data indicate linearity across a large dynamic range of 10–1000 ng/ml for all five LCS (Table). For each LCS, LOD was below 0·3 ng/ml while LOQ was below 0·7 ng/ml. Accuracy and precision were tested by analysing spiked urine samples at three concentrations within the dynamic range (ie.12·5, 562·5 and 930 ng/ml).

Sweetener	Linearity (R^2)	Accuracy[a] (%)	Precision[a] (%CV)	
			Within run	Inter-day[b]
Acesulfame-K	0·999	98–112	1·4–1·9	1·4–4·9
Cyclamate	0·999	97–101	0·9–2·3	0·4–3·8
Saccharin	0·999	99–115	0·3–3·1	2·0–10·7
Steviol glucuronide	0·997	94–111	0·9–2·3	3·1–7·5
Sucralose	0·996	98–106	1·3–8·7	7·7–8·9

[a] Triplicate runs carried out at each concentration; values represent the range between concentrations. [b] Results of analyses undertaken over 3 consecutive days

This novel method represents an opportunity to implement a biomarker approach for objectively assessing dietary exposure to five LCS. Further work is underway to establish the level of sensitivity of the method within the expected range of LCS observed in free-living populations.

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