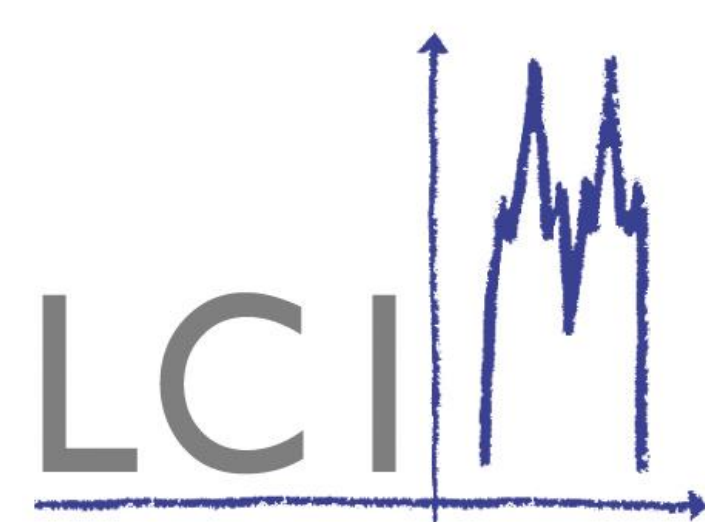


# Quick Analytical Method for determining selected Polyphenols in Cocoa and Chocolate Products



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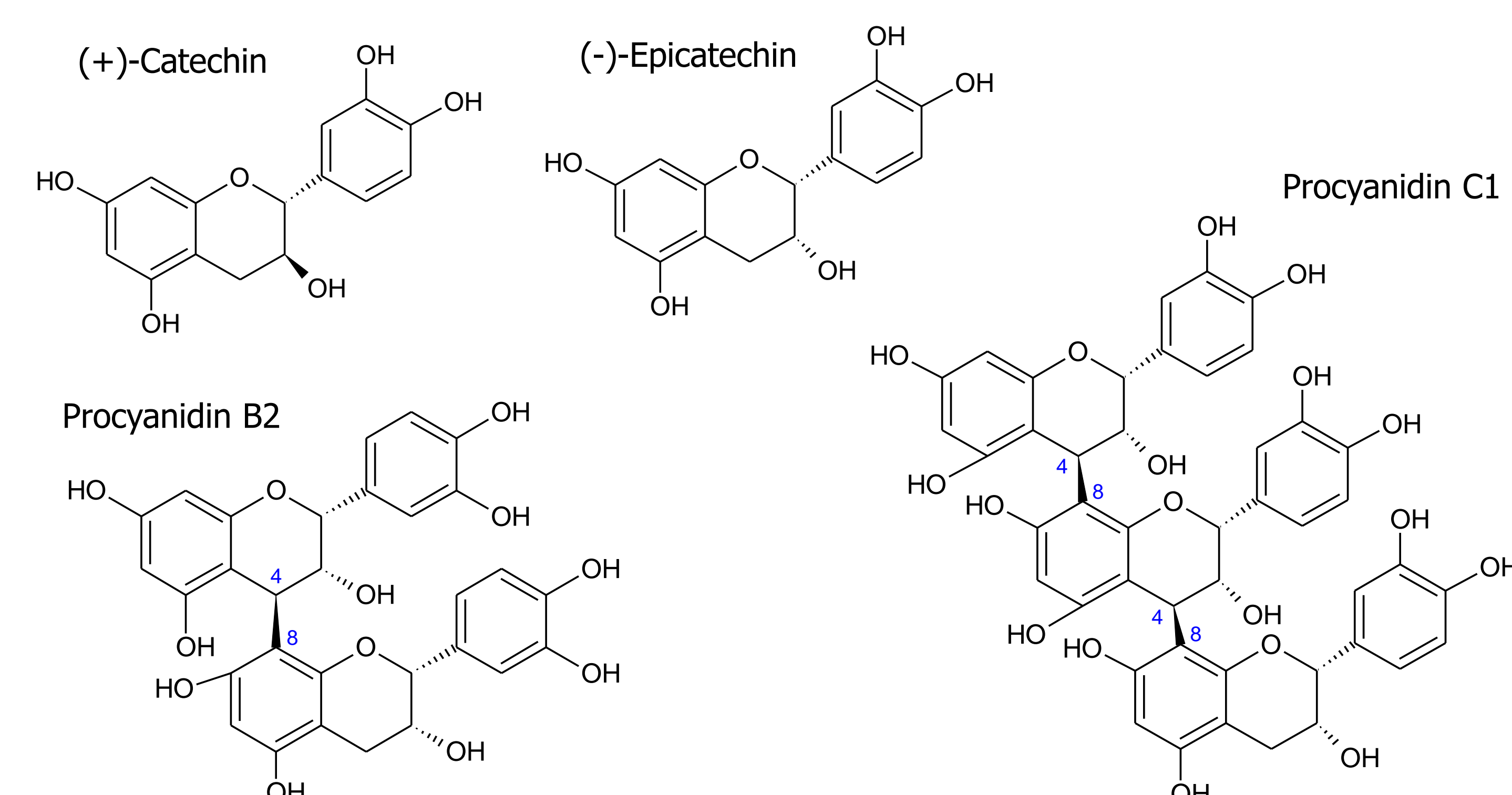
## Background

Polyphenols are, quantitatively speaking, one of the key classes of naturally occurring compounds – they are produced by all plants, without exception, as secondary plant substances (secondary metabolites). Although polyphenols are non-nutrient compounds, they have a positive effect on human health thanks to their antioxidant property. This explains why they have increasingly attracted scientific, physiological, technological, and commercial interest over the last decade.



Polyphenols can be put into three classes: the phenolic acids, the tannins, and the flavonoids. The latter play a key role, especially in cocoa. Quantitatively speaking the monomer flavanols (-)-epicatechin, (+)-catechin, and various highly polymer procyanidins are dominant and are particularly responsible for the bitterness and astringency but also the typical colouring [1-3].

## Polyphenols



## Validation

The presented method was validated based on the guidelines provided by Kromidas [4]. For this purpose a chocolate sample was prepared and analysed according to the method developed within the scope of this study. Table 1 shows a summary of the key validation results, such as the relative standard deviation of repeatability ( $RSD_r$ ) and reproducibility ( $RSD_R$ ), the recovery rate, and the limit of detection (LOD) and limit of quantification (LOQ) of the four examined flavonoids.

Table 1: method validation results

	Catechin	Epicatechin	Procyanidin B2	Procyanidin C1
Repeatability $RSD_r$ [%]	5.1	4.6	5.5	5.8
Reproducibility $RSD_R$ [%]	8.1	5.3	5.9	8.2
Recovery rate [%]	75.5	81.4	n.d.	n.d.
LOD [ $\mu\text{g/g}$ ]	5	6	3	8
LOQ [ $\mu\text{g/g}$ ]	15	17	9	24

(n.d. = not determined)

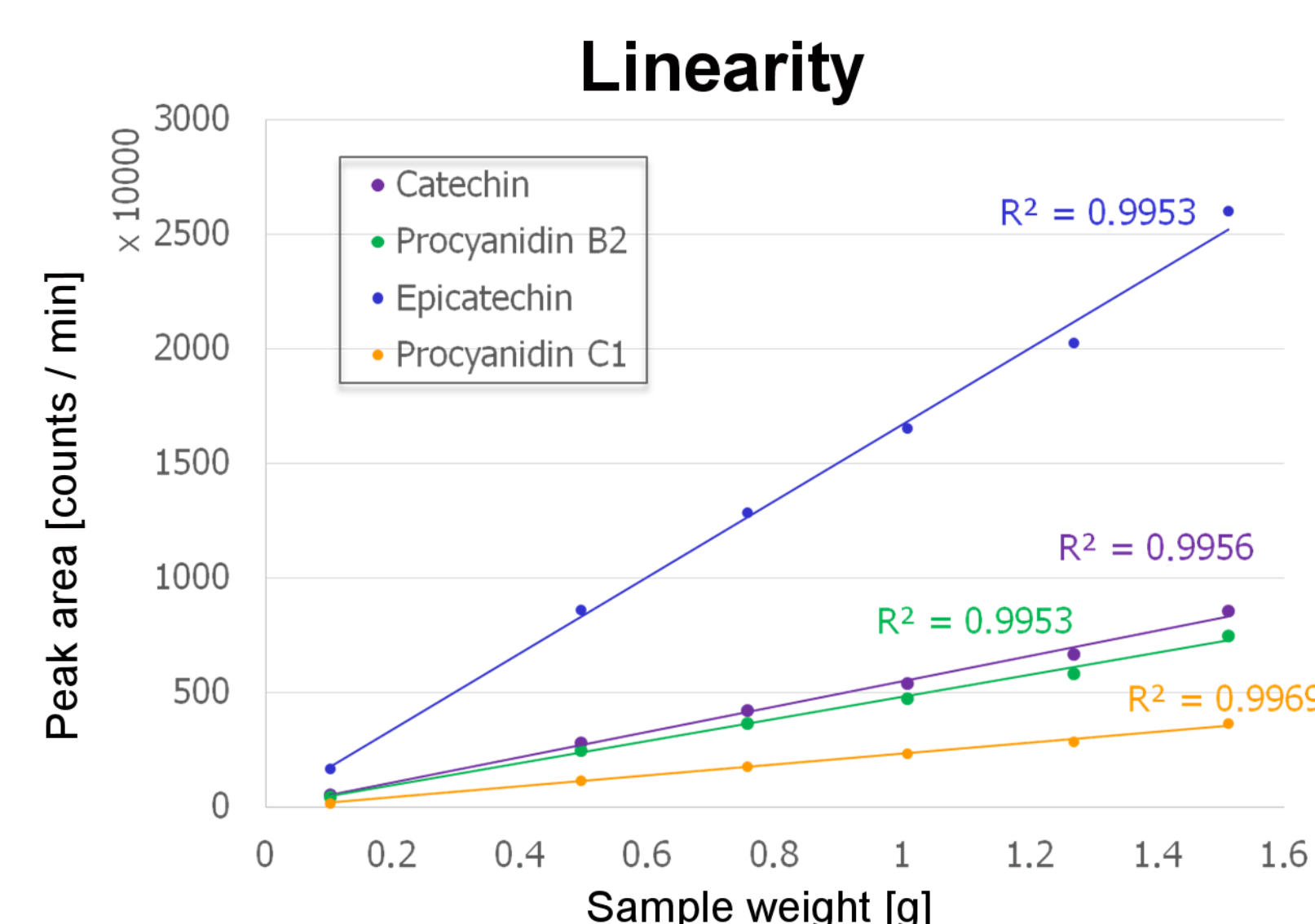


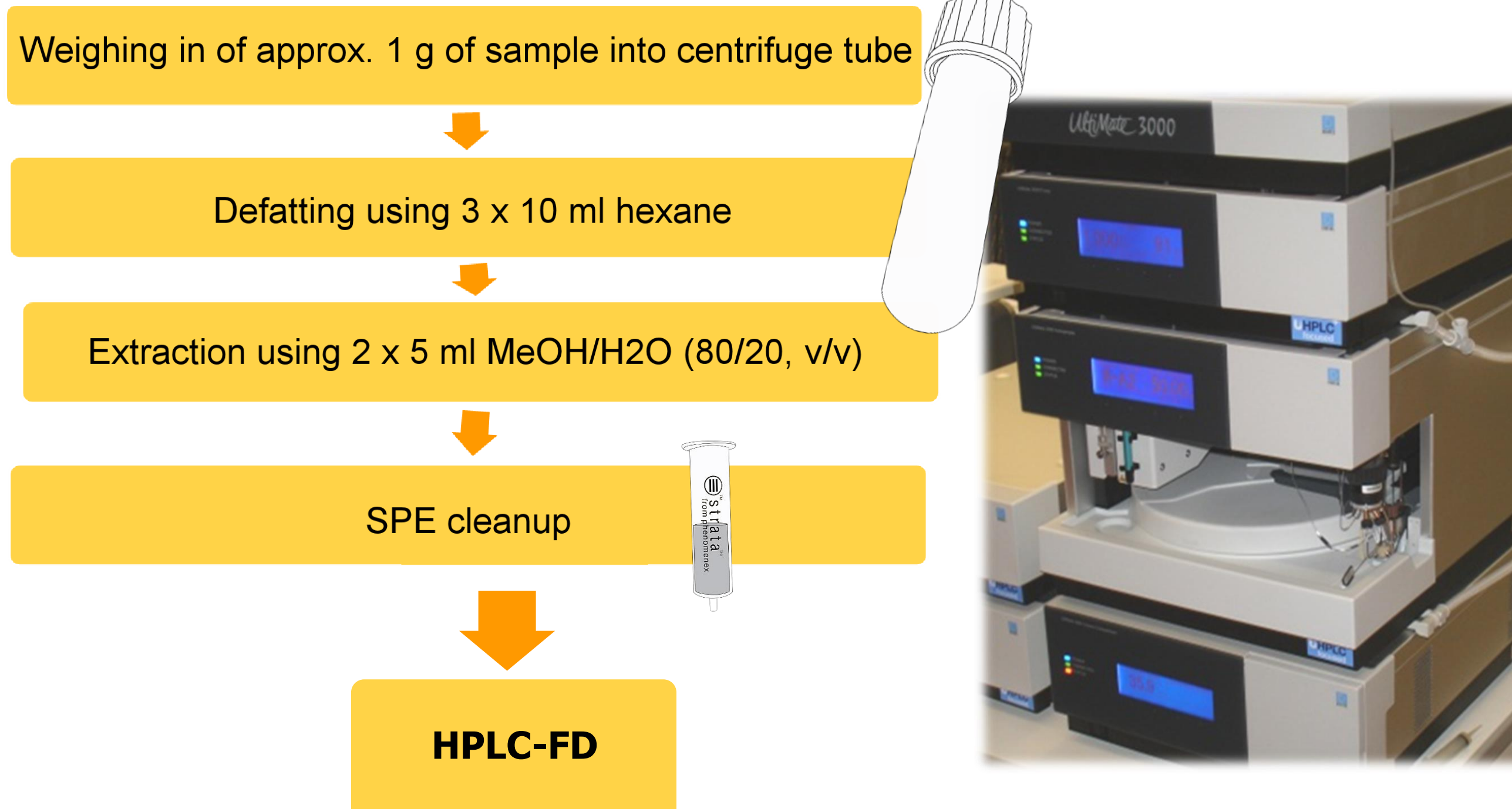
Fig. 1: linearity check for examined flavonoids

Figure 1 shows the linearity check for the flavonoids catechin, epicatechin, procyanidin B2 and procyanidin C1. This produced a very satisfactory coefficient of determination ( $R^2$ ) of at least 0.9953 in the examined sample weight range of 0.1 to 1.5 g for the four flavonoids under analysis here. Hence the method presented here is linear over a broad concentration range, has a good reproducibility, and – with a limit of detection (LOD) of between 3 and 8  $\mu\text{g/g}$  – is very sensitive.

## Objective

Our research was aimed at establishing and validating a quick analysis method for determining select polyphenols in cocoa and chocolate products using HPLC-FD. Quantification of the flavonoids epicatechin, catechin, procyanidin B2, and procyanidin C1 in the samples was performed using external standards.

## Sample Processing



## HPLC Parameters

- Column: ultra PFPP (100 x 2.0 mm; 3  $\mu\text{m}$ )
- Oven temperature: 30°C
- Injection volume: 1  $\mu\text{l}$
- Eluent: A: 0.1% formic acid  
B: 0.1% formic acid in acetonitrile
- Fluorescence detection: excitation at 280 nm, emission at 318 nm
- Mode: gradient (cf. Figure 2)

Figures 3 and 4 show the HPLC-FD chromatogram of the mixed standard containing the flavonoids (+)-catechin, (-)-epicatechin, procyanidin B2 and C1 and a chocolate sample respectively.

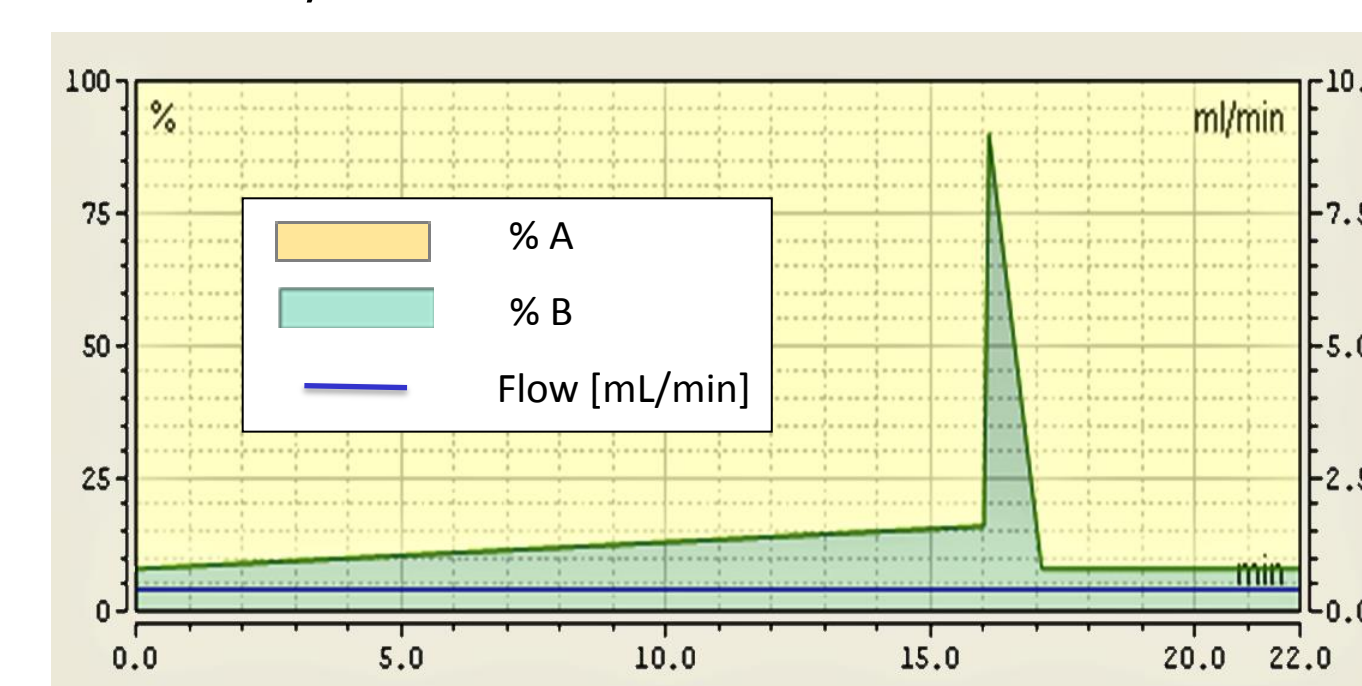


Fig. 2: gradient programme and flow rate (0.45 ml/min)

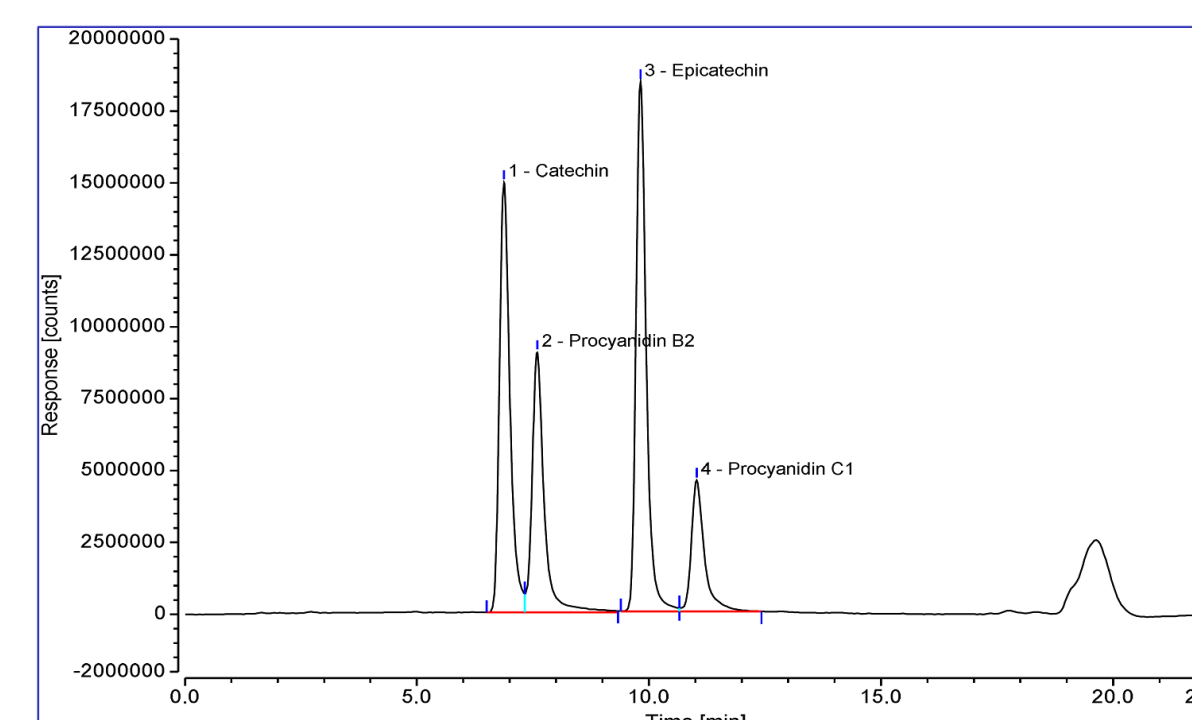


Fig. 3: mixed standard (20  $\mu\text{g/ml}$  each of (+)-catechin, procyanidin B2 and procyanidin C1, 30  $\mu\text{g/ml}$  (-)-epicatechin)

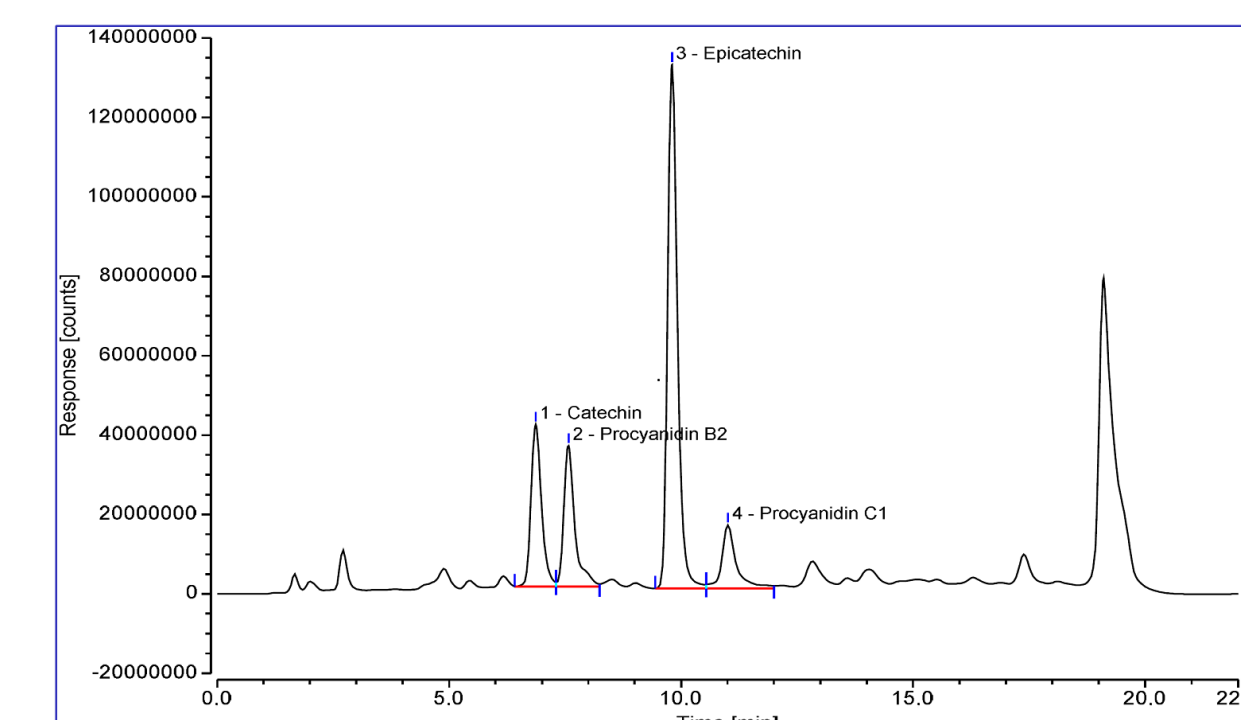


Fig. 4: chromatogram of a chocolate sample (content levels [mg/g]: catechin=0.25; procyanidin B2=0.36; epicatechin=1.01; procyanidin C1=0.35)

## Conclusion

A quick and simple analytical method for determining select polyphenols (catechin, epicatechin, procyanidin B2 and procyanidin C1) in cocoa and chocolate products has been successfully established and validated.

This method is a suitable procedure for routine analysis. After only a little preparation, the sample extract can be analysed within a short time using HPLC-FD and the flavonoids contained in the sample can be qualitatively and quantitatively determined via external standards.

## References

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